# CYTOLOGICAL AND GENETICAL STUDIES

BY

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## Introduction

Most of the important contributions which made the foundation of cytogenetics, were obtained by studying chromosome behaviour in plants. It is however of primary importance to demonstrate the universal validity of those laws which underlie chromosome behaviour in animals as well as in plants. Animals are less suitable for detailed cytological analysis , partly owing to technical difficulties, and partly to the great number and small size of the chromosomes in most animal species. It was found that in the animal kingdom the order of Orthoptera is one of the groups containing species which offer excellent material for cytological investigations. Since Wenrich ( I9I6, I9I7), who established the fact of chromosome individuality in Phrynotettix, a number of observations have been made on Orthoptera. Thus Janssen's ( I924 ) important investigations which gave a cytological basis to the phenomenon of genetical crossing-over were carried out on grasshoppers. Darlington and Dark ( I932 ) built up their electrostatic theory of chromosome association by analysing chromosome behaviour in Stenobothrus. Quite recently , the complexity of crossing-over was explained and its cytological interpretation given by Darlington in his studies on Chorthippus and Stauroderus ( I936 ).

These few but important contributions clearly show that further studies on species belonging to Orthoptera may prove to be very fruitful. With this view, cytological analysis was undertaken in three genera of Orthoptera: Two of these, Chondracris and Oxya , had not been studied cytologically before. In the third genus, Locusta, chromosome behaviour was studied with a view to obtaining data for a future analysis regarding the effects of geographical distribution on chromosome structure and behaviour.

## Material and Technique

Three species of the family Acrididae, namely , Locusta migratoria L., Chondracris rosea de Geer , and Oxya velox Fabr., were investigated. Locusta is a short-horned locust widely distributed in Asia, Europe, and Africa. Chondracris and Oxya are solitary grasshoppers found only in certain parts of Eastern Asia; China, Formosa, and Japan. The animals were caught on August 26th, 1936, in the surburbs of Peiping. Their testes were fixed in medium Flemming. Sections were cut at a thickness of 25$\mu$ and stained with gentian violet. The drawings were made by using an 1.2 apoch. oil-immersion objective and X 20 compensation eye-piece. The magnification is given in the legends.

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## Chromosome Number and Morphology

In Locusta and Chondracris, numerous spermatogonial cells showing various stages of mitosis were found, while in Oxya their number was very few. In all three genera, the chromosome number was found to be 23, one of which is the sex chromosome. Three types may be distinguished, long, medium-sized, and small dot chromosomes ( fig. 1 ). The long chromosomes are about 6——8μ in length; the medium-sized chromosomes measure 4—6μ and the dot chromosomes are 2—3 times smaller than the medium-sized.+ The number of different chromosomes varies in the three genera; their distribution is given in table 1. It is generally accepted that the shape of the chromosome is determined by the position of the centromere. Since all the chromosomes in the three genera are rod-shaped ( except the dot chromosomes ), the position of the centromere is believed to be terminal or nearly so. At mitotic prophase, the chromosomes show distinct relic spirals; their number in a particular chromosome was found to be three of four. In all three genera the chromosomes during metaphase are arranged in a more or less complete ring, with the long chromosomes at the periphery and the small dot chromosomes in the center. In Oxya, the spermatogonial cells are relatively small and the axis of the

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+ White ( 1935 ) illustrated the chromosome complement


Fig. 1. Mitotic metaphase; (a) Chondracris ,
(b) Locusta, (c) Oxya. X 2500

Table 1

Chromosome Types

| Genus | Long | +Medium | | Short | Total no. of 2n, |
|---|---|---|---|---|---|
| | | $M_1$ | $M_2$ | | |
| Locusta | 2 | 2 + X, | 4 | 3 | 22 + X |
| Chondracris | 2 + X, | 4 | 2 | 3 | 22 + X |
| Oxya | 2 | 4 + X, | 3 | 2 | 22 + X |

+ The chromosomes which are about 5—6$\mu$ in length are classified as $M_1$; the others, which are less than 5$\mu$, are grouped into class $M_2$.

spindle is short. The sex chromosome in respect of length belongs to the L chromosome group in Chondracris and to $M_1$ in Locusta and Oxya ( table 1 ). It does not show precocity during mitotic prophase, but it can be distinguished at metaphase because it has no partner.

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in Locusta migratoria , and there is a close agreement between his and our observation.

## Chromosome Behaviour and Chiasma Frequency during Meiosis

During the early prophase of meiosis ( leptotene ), the chromosomes emerge as single threads from various parts of the nucleus. In Locusta, there is pre-pachytene 'polarization'; the chromosome threads emerge from the periphery of the nucleus and spread out into the central region. Such an arrangement is due to the position which the chromosomes occupy at the end of the previous spermatogonial division. Soon the threads begin to thicken, and homologous chromosomes come to associate with each other in different regions ( zygotene ). Their complete association is sometimes prevented by interlocking with non-homologous chromosomes, as frequently seen in Oxya, but usually the pairing of homologues is complete ( pachytene ). The number of paired threads within the nucleus of the three genera is found to be eleven.

The sex chromosome was identified as a large nucleolus-like body, commonly lying at random among the other paired threads. Besides this nucleolus-like body, which is present in all three genera, there was found in Oxya a nucleolus in stricto sensu attached to 2 chromosome pairs. The size of the nucleolus is about the same as that of the nucleolus-like body.

After pachytene, there is a stage when the paired

chromosome threads lose their staining capacity. This stage is very short, and was shown by only very few spermatocytes. The onset of diplotene is characterized by the appearance of small loops in the bivalents. In Locusta there are usually two loops in a long bivalent and one in a medium-sized during late diplotene ( fig. 2 ); in Chondracris ( fig. 3 ) and Oxya ( fig.4 ) the number of chiasmata is fewer. The loops are assumed to be the result of the exchange between partner chromatids of the homologous chromosomes; the nodes represent the chiasmata, or the loci where exchange has occurred ( Darlington , 1932 ).

At diakinesis, the bivalents are short and thick, indicating a strong internal contraction ( fig. 5.a, b; 6. a, b; 7. a, b, ). While they are close together at diplotene, they are separated during diakinesis, owing to a strong interchromosomal repulsion. In Oxya ( fig. 6. a, b ) the distribution of the bivalents in the cell is quite regular. In Chondracris ( fig. 5. a, b ) some bivalents are grouped more closely together while others are pushed off to one side, indicating a differential effect in the repulsion,due possibly to the difference in size of the bivalents.

The chiasmata during diplotene are distributed in

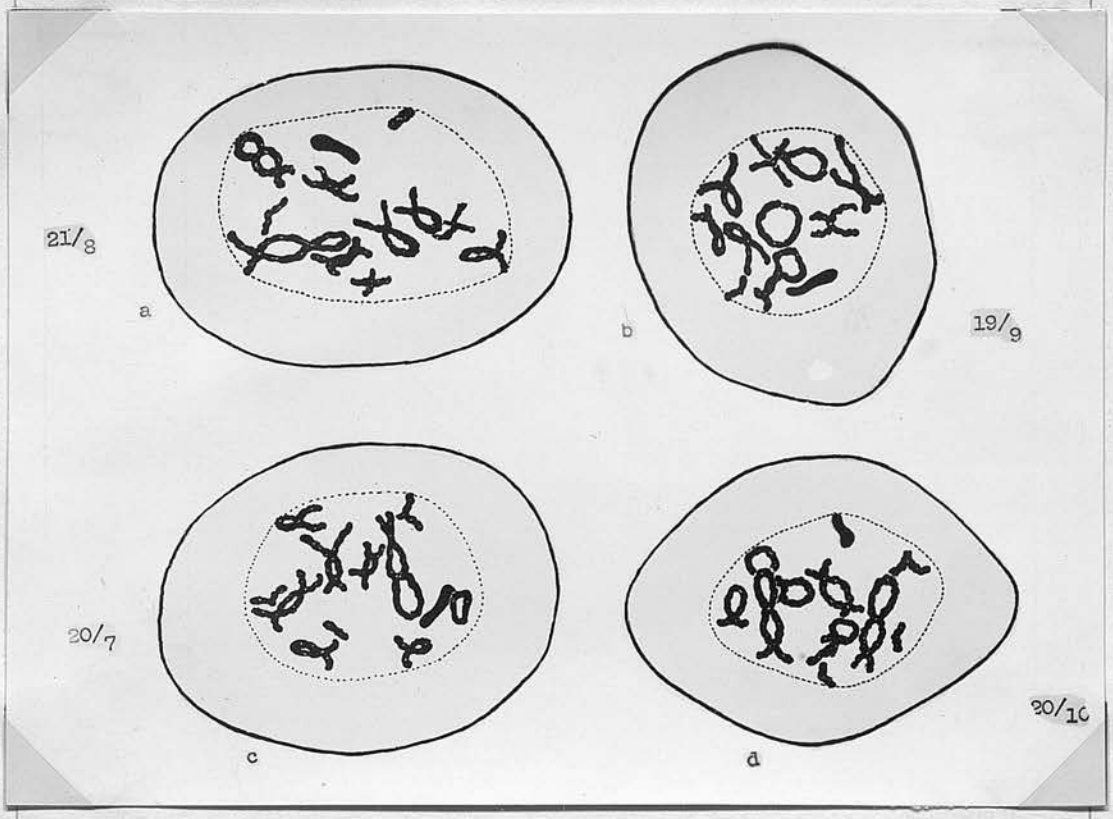



Fig. 2. (a), (b), (c), (d), Late diplotene in Locusta. The sex chromosome shows precocious condensation. The total number of chiasmata and terminal chiasmata per nucleus is given for each cell. X 2300

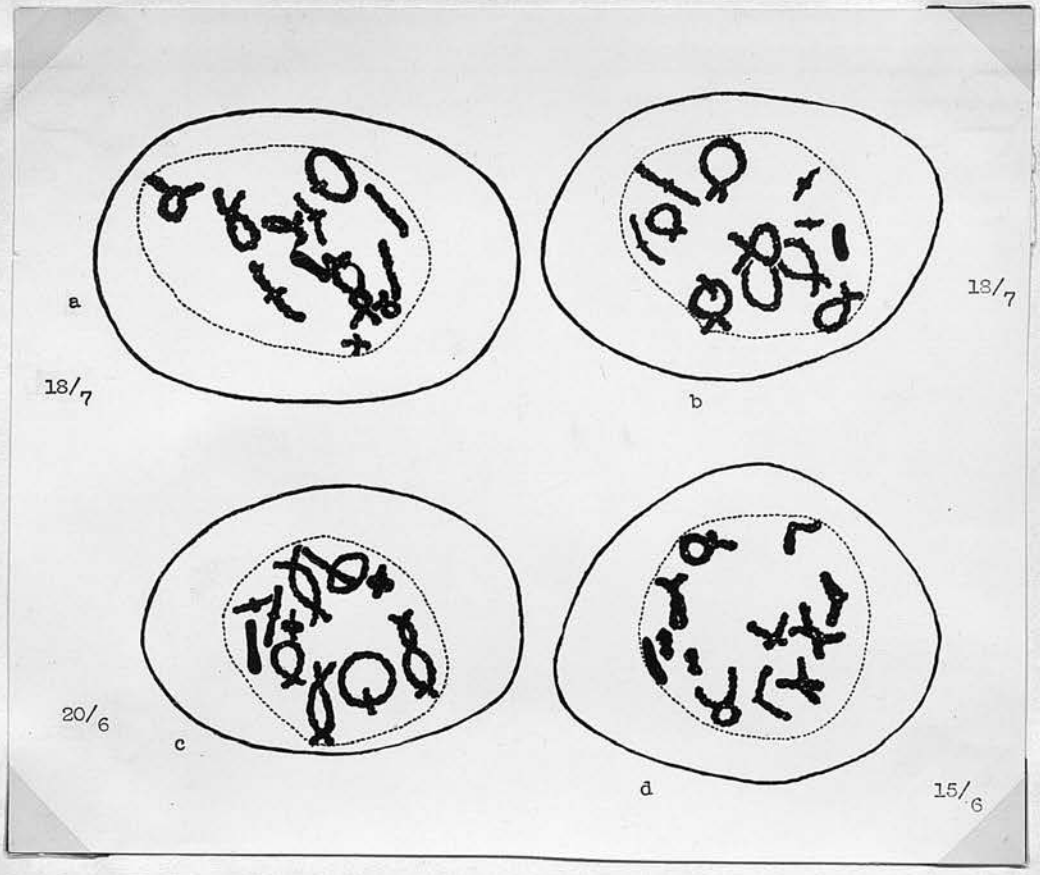



Fig. 3. (a), (b), (c), (d), Late diplotene in Chondracris. ×2300

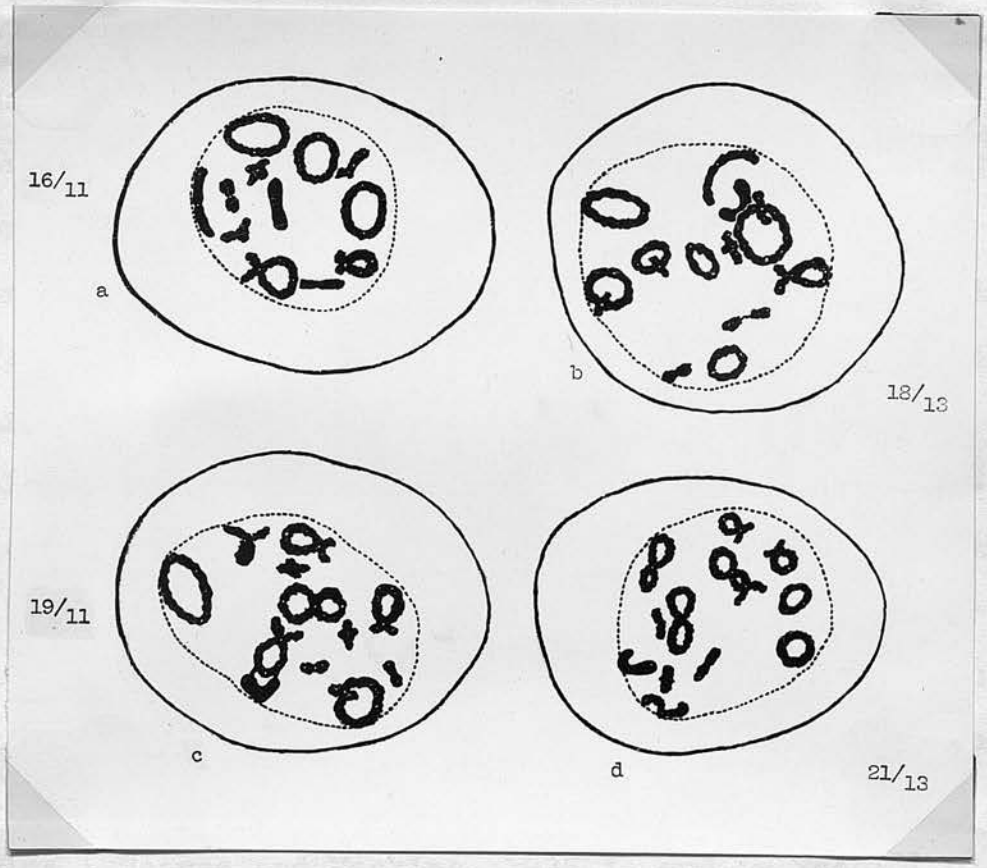



Fig. 4. (a), (b), (c), (d), Late diplotene in Oxya. The sex chromosome is attached to a bivalent in (c). ×2300

the bivalents at random except in Oxya, where some bivalents show chiasmata near the ends, indicating a certain degree of localization. The number of chiasmata bears some relationship to the length of the chromosomes. It was found that the number of chiasmata in the long bivalents is higher than in the short ones. This relationship however is not a direct one: the number of chiasmata does not increase in proportion to the increase in length. The small bivalents have one chiasma, and the medium-sized, which are two or three times longer, have two or three , showing a direct relationship; but the longest bivalents, which are about four or five times longer than the shortest, contain no more chiasmata than the medium-sized bivalents. Similar relationship between chromosome length and chiasma frequency was found in Stenobothrus ( Darlington and Dark, 1932 ), Melanoplus ( Hearne and Huskins, 1935 ), and in Yucca ( O' Mara, 1931 ). According to Darlington ( 1937 ), in these species the chiasma frequency is no longer a direct function of length, and the indirect proportionality is a genetical adaptation, necessary for the pairing of short chromosomes.

The number of chiasmata in all three genera is decreased at diakinesis. This is due to movement of the chiasmata away from the centromere ( Darlington,

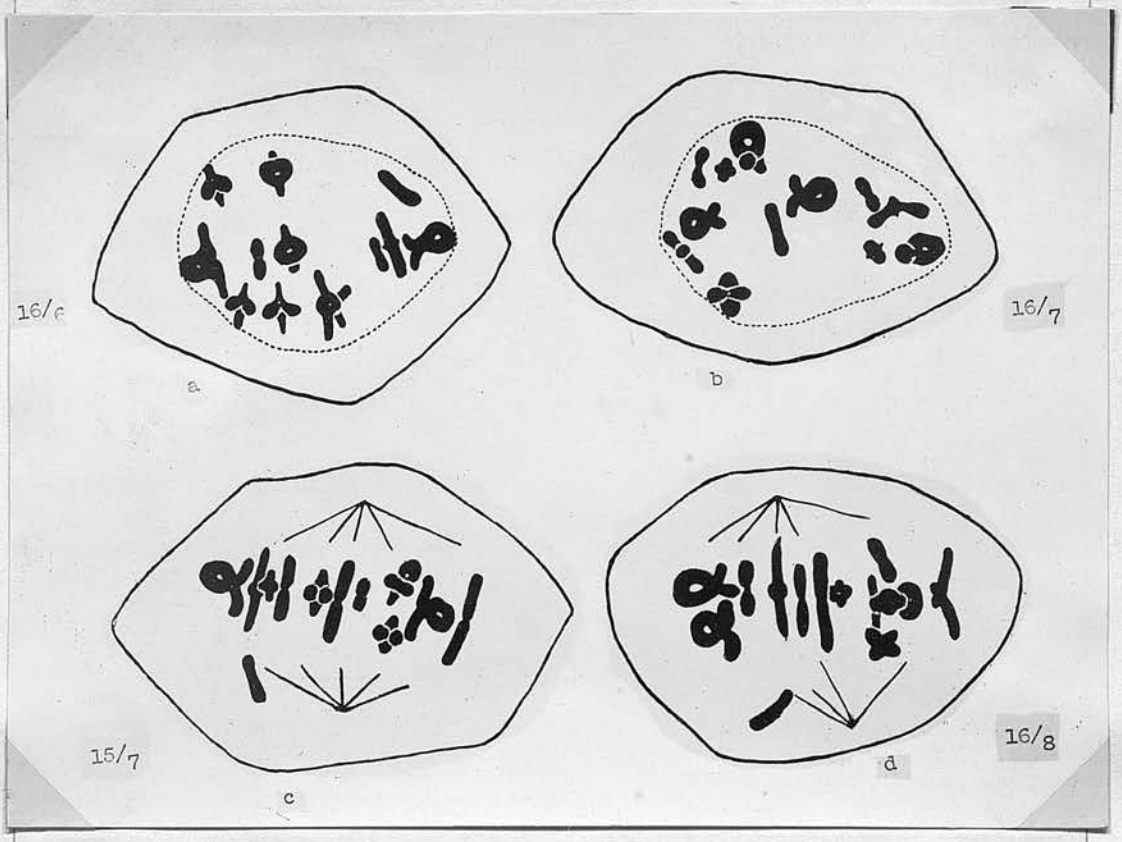



Fig. 5. (a), (b), Diakinesis; (c), (d), metaphase in Chondracris. The sex chromosome is lying off the equatorial plate during metaphase. X2300

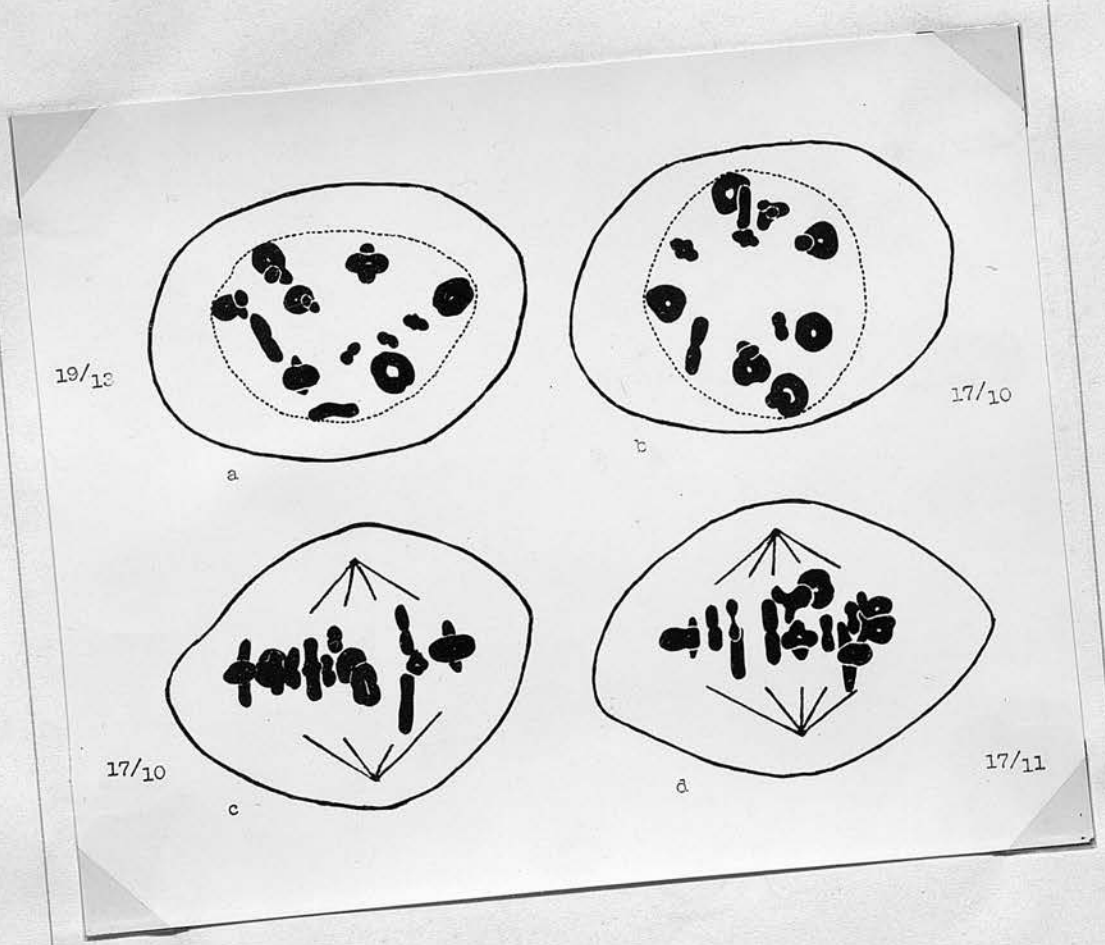



Fig. 6. (a), (b), Diakinesis; (c), (d), metaphase in Oxya. ×2300

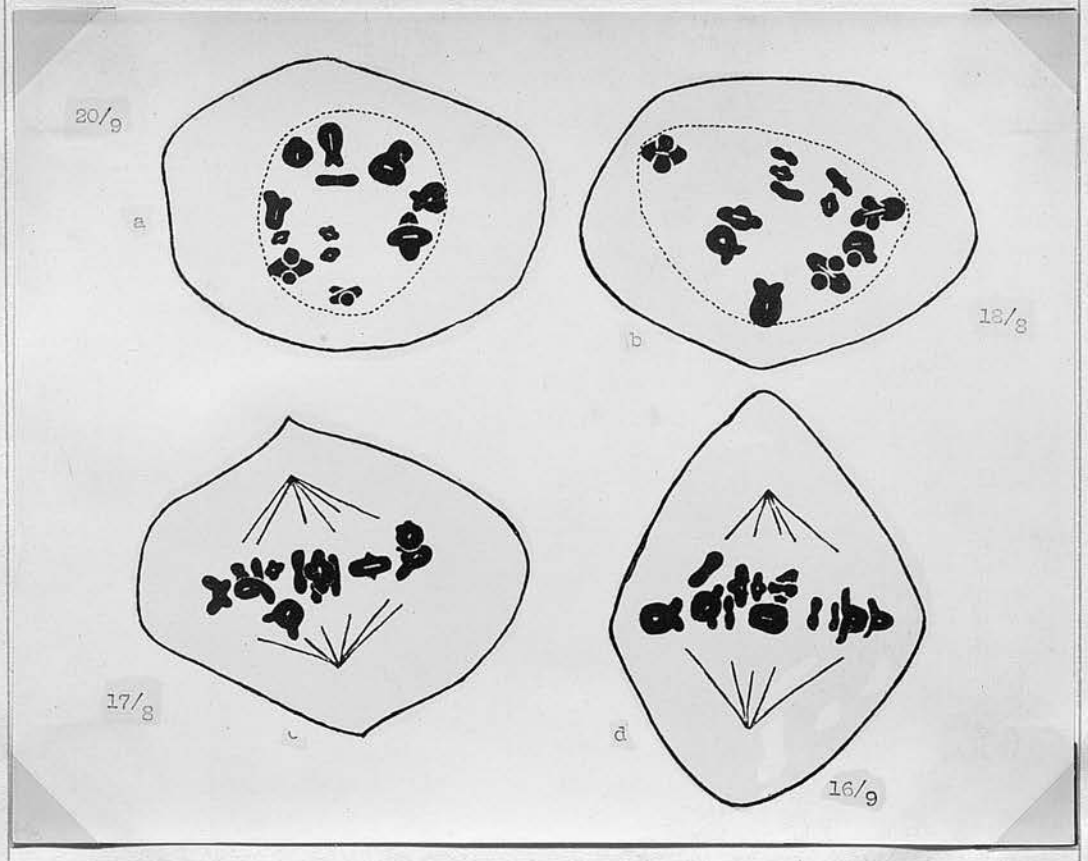



Fig. 7. (a) , (b), Diakinesis; (c), (d), metaphase in Locusta. X 2300

1932 ). The force of repulsion which operates between partner chromosomes pushes the chiasmata towards the distal end because the general repulsion, which is present all along the chromosome, is increased at the region of the centromere, owing to the specific repulsion operating between centromeres ( Darlington, 1937 ).

Diakinesis is followed by a stage which may be termed pro-metaphase. It is characterized by the disappearnce of the nuclear membrane, without any definite orientation of the bivalents on the equator. The spindle is not yet formed. This stage is relatively long in Oxya, but short in Locusta and Chondracris, as indicated by the number of spermatocytes showing it. The bivalents are more contracted and the number of chiasmata further decreased. A comparison of this stage with the following metaphase shows that after the pro-metaphase there is no further contraction within the bivalents and no further reduction in the number of chiasmata.

The bivalents during metaphase are arranged on the equatorial plane ( fig. 5, 6, 7 ). The spindle is well-developed and may be clearly seen in Locusta. The distal segments of the bivalents in some spermatocytes were lying outside the spindle region ( fig. 7. c, d ). One or sometimes two bivalents

were found lying off the equator, forming a secondary or accessory plate. Such an arrangement is due to crowding of the bivalents on the metaphase plate ( Darlington, 1937 ).

The distance between the centromeres and the centrosomes is nearly the same for all bivalents irrespective of size. In Oxya ( fig. 6, c, d ) several meiotic metaphase plates were encountered where the short bivalents were nearly separated; their members were connected with a long thin terminal thread, so that their centromeres were lying at the same level as those of the other bivalents. In polar view , it can be seen that the bivalents are arranged in a large circle, within which are located the small bivalents with terminal chiasmata. The polar view of metaphase shows that the large ring bivalents have two chiasmata; one is subterminal and is localized at the centromere region, while the locus of the other is distal-terminal.

The chiasma frequency during prophase and metaphase was counted in the three genera, and is given in table 2.

It can be seen that there is a definite decrease in the number of chiasmata from diplotene to metaphase in the three genera. This decrease between the three stages is of the same rate in Locusta and

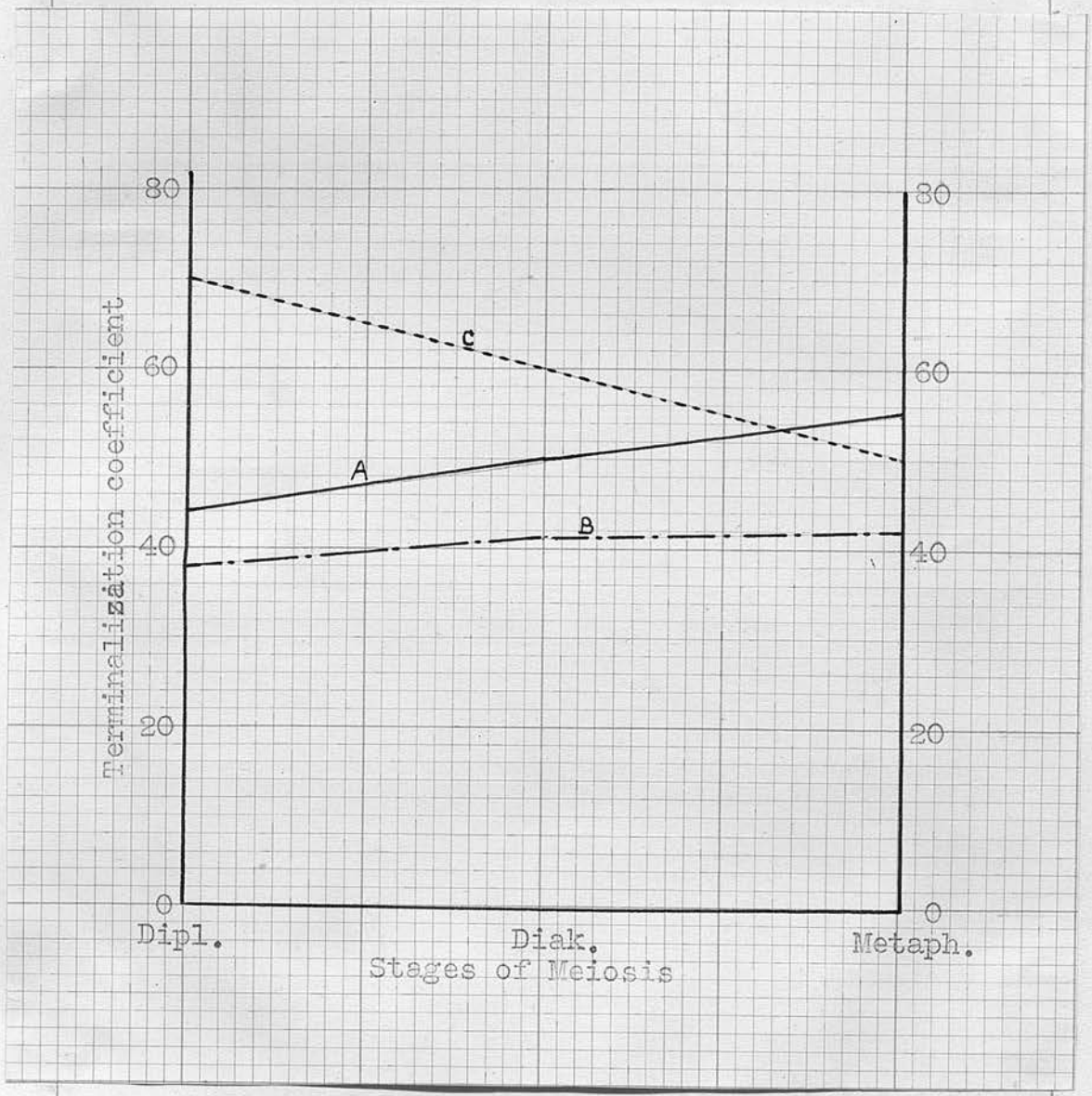
Table 2.— Chiasma Frequency in the three Genera.

| Genus | Stage of Meiosis | No. of Nuclei | No. of bivalents | No. of bivalents with 1Xta | 2Xta | 3Xta | Total no. of Xta | Total no. of Term. Xta | X./ Bivalent | Xta/ Nucleus | Term. Coef. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| L. | Dip. | 25 | 275 | 100 | 130 | 45 | 495 | 221 | 1.8 | 19.8 | .44 |
| | Dia. | 25 | 275 | 130 | 124 | 21 | 441 | 222 | 1.6 | 17.6 | .50 |
| | Met. | 25 | 275 | 164 | 108 | 3 | 389 | 213 | 1.4 | 15.5 | .55 |
| C. | Dip. | 25 | 275 | 128 | 131 | 16 | 438 | 170 | 1.5 | 17.5 | .38 |
| | Dia. | 25 | 275 | 151 | 115 | 9 | 408 | 167 | 1.4 | 16.2 | .41 |
| | Met. | 25 | 275 | 188 | 77 | 10 | 372 | 155 | 1.3 | 14.8 | .42 |
| O. | Dip. | 16 | 176 | 64 | 90 | 22 | 310 | 219 | 1.7 | 19.3 | .70 |
| | Dia. | 25 | 275 | 122 | 133 | 20 | 448 | 269 | 1.6 | 17.9 | .60 |
| | Met. | 16 | 176 | 83 | 89 | 4 | 273 | 141 | 1.5 | 17.0 | .51 |

( In the above table, L. stands for Locusta, C. for Chondracris, and O. for Oxya. )

Chondracris, but it is smaller between diakinesis and metaphase in Oxya. The average chiasma frequency per bivalent, irrespective of size, in the three genera is between 1.3 and 1.5 during metaphase, but when counts were made for the short and the long bivalents separately, it was found that the chiasma frequency in the small bivalents of the three genera is 1, while in the long bivalents it is much higher, i. e. in Locusta it is 1.8, in Chondracris 1.6, and in Oxya 1.9.

The proportion of terminal chiasmata to the total number of chiasmata is indicated by the terminalization coefficient. It is calculated by dividing the number of terminal chiasmata by the total number of chiasmata, and may be taken as a measure of the movement of chiasmata away from the centromere. The terminal coefficients in the three genera are illustrated in graph 1. There is an increase of terminal chiasmata from diplotene to metaphase in Locusta and Chondracris, but a definite decrease in Oxya. This difference may be due to a difference in the specific behaviour of the terminal chromomeres of the chromosomes. The specific affinity present in the terminal chromomeres is probably different in different species. It may also depend on environmental causes. From




Graph.1. The terminalization coefficients in the three genera; A: Locusta, B: Chondracris, C: Oxya.

the above data, we may assume that Oxya has a low affinity of terminal chromomeres as compared with the other genera.

After metaphase, the members of bivalents separate and segregate towards opposite poles. In Locusta, it was seen that bivalents with two chiasmata sometimes lag at first anaphase and their separation is delayed. It is very probable that such bivalents have disparate chiasmata and chromatid interlocking ( Moffett, 1932. ). The first meiotic anaphase is followed immediately by the second metaphase without an interphase. The chromatids are seen very clearly; sometimes they are wide apart at the distal region. The small chromosomes in the three genera are arranged at the centre of the metaphase plate while the longer ones are located at the periphery, only the centromeres being orientated on the plate ( fig. 8, 9, 10 ) . There is a causal connection between the arrangement of chromosomes at the first and at the second metaphase. During the first metaphase, a polar view shows that the small bivalents are at the centre of the equatorial plate, and they occupy the central region of the equatorial plate during the second metaphase also. The second anaphase is very short, as it was shown by only a few secondary

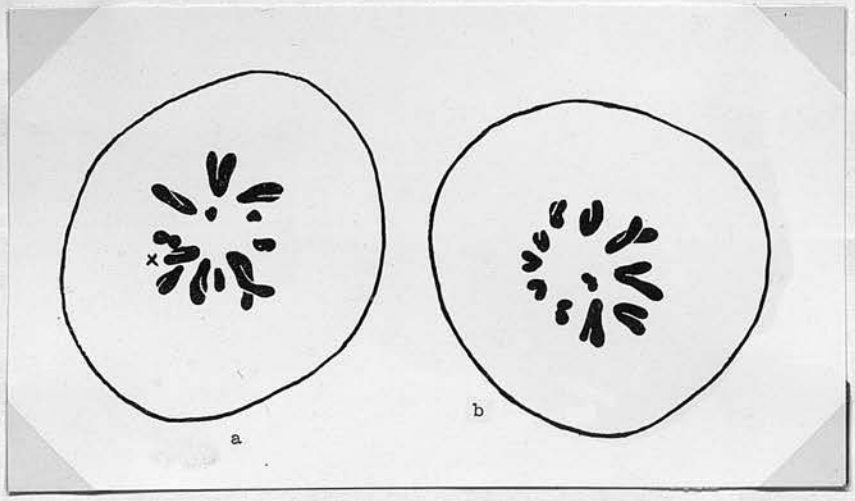



Fig. 8. Second metaphase in Oxya; (a), with the X-chromosome, (b), without the X-chromosome. X2300

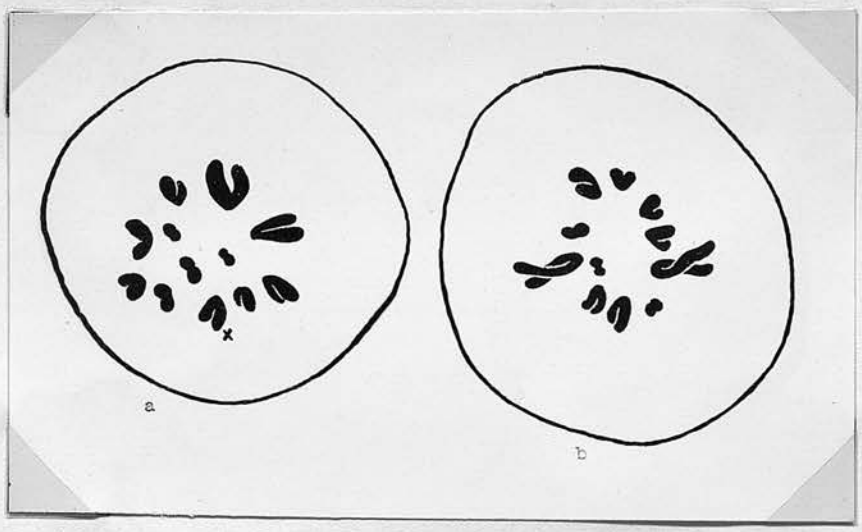



Fig. 9. Second metaphase in Locusta; (a), 11 chromosomes + X, (b), 11 chromosomes. X2300

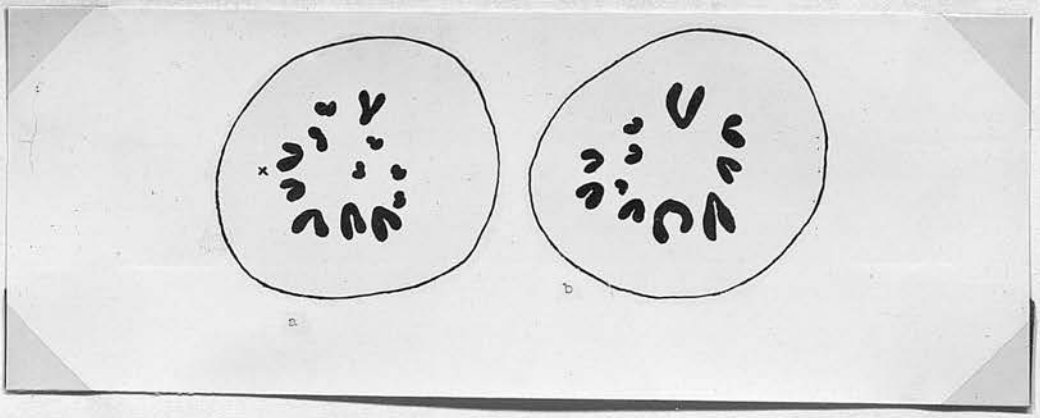


Fig. 10. Second metaphase in Chondracris; (a), 11 chromosomes + X , (b), 11 chromosomes. X2300

spermatocytes. The chromosomes which have divided equationally now move towards opposite poles. After a short telophase, they lose their staining capacity and after the formation of a nuclear membrane cannot be identified any more. The spermatids contain a well-defined round nucleus showing one or two nuclear aggregates lying amongst a fine network.

Behaviour of the Sex Chromosome during Meiosis.

During the prophase of meiosis in the three genera, the X chromosome is present as a more or less distinctly stained nucleolus-like body. Its shape is oblong or sometimes crescent-formed. In Locusta , it is associated with one chromosome pair; in Oxya, with two chromosome pairs. In Locusta, polarization was occassionally found during pachytene , and in such a nucleus the sex chromosome lies near the attraction region, towards which the ends of the autosomal chromosomes are turned. At the end of pachytene, the sex chromosome stains more deeply : this may be a measure of the internal contraction . During diplotene, it appears as a thick rod-shaped body ( fig. 2, 3, 4 ) which in a few instances is still connected with one or two bivalents. During diakinesis, it shows the same contraction and condensation as the other chromosomes ( fig. 5, 6, 7 ). In a few primary spermatocytes, it was seen that during this stage the sex chromosome is double, containing two chromatids lying side by side without relational coiling.

At meiotic metaphase, the sex chromosome very frequently lies near one pole ( fig. 6, c; 7, d ) and sometimes it is placed outside the spindle, as in Chondracris ( fig. 5, c,d ). The characteristic

position of the sex chromosome may be taken as an indication that this chromosome differs from the autosomal bivalents. This difference may be qualitative, i. e. the sex chromosome may have quite a different surface charge ( Koller and Darlington, 1934 ) as a result of its internal organization. However, it is not improbable that the position of the sex chromosome may be influenced by its single or unpaired nature. In this respect, it shows behaviour somewhat similar to that of univalent chromosomes in various organisms.

At anaphase, the sex chromosome usually proceeds to the nearer pole without dividing. In all three genera, the first meiotic division is always reductional in respect of the sex chromosome. The chromosome number during the first anaphase and the second metaphase indicates that the pre-reductional segregation of the sex chromosome is obligatory. Two kinds of metaphase plate were found in equal numbers, one with eleven ( fig. 8 b, 9 b, 10 b ), and the other with twelve chromosomes ( fig. 8 a, 9 a, 10 a ). The fate of the sex chromosome in the following stages could not be followed. The fact that the distribution of the nuclear aggregates within the spermatids is variable, suggests that the sex chromosome may form a visible structure and that it may be pos-

sible to distinguish the spermatids which carry it. However, it was not possible to verify this assumption owing to insufficient material.

Summary

1. Three genera of the family Acrididae, Chondracris and Oxya, indigenous groups of East Asia, and Locusta, which is a widely distributed group, are investigated. The diploid chromosome number is 23, namely, eleven pairs of autosomes and one sex chromosome, in all three genera.
2. The chromosomes are of three types as regards the length: long, 6 — 8μ; medium, 4 — 6μ; and short which are about two or three times smaller than the medium ones. They are either dot- or rod-shaped, indicating that the centromere is terminal.
3. In Locusta, there is pre-pachytene 'polarization'; the chromosomes emerge from the periphery of the nucleus and spread into the central region. Pachytene polarization is also observed in Locusta, while it is absent in the other two genera.
4. The distribution of the chiasmata during diplotene is at random. In Oxya, some bivalents have partially localized chiasmata near the ends.
5. The number of chiasmata shows a direct relationship with length in the short and medium-sized bivalents of the three genera, whereas the long

bivalents have fewer chiasmata per unit of length.

6. There is a relatively slight decrease in chiasma frequency from diplotene to metaphase in all three genera.

7. In Locusta and Chondracris there is an increase of the terminalization coefficient from diplotene to metaphase; in Oxya, the terminalization coefficient is decreased during these stages. The cause of this difference in behaviour may be either genetical or environmental.

8. The X chromosome appears in the nucleus during pachytene as a nucleolus-like body which is attached to other chromosomes and distinctly stained. Its shape is oblong or crescent-formed in all three genera. It stains more deeply at the end of pachytene but at diakinesis shows the same degree of condensation and contraction as the other chromosomes.

9. The X chromosome frequently forms an accessory plate during metaphase in all three genera. At the following anaphase, it segregates towards the nearer pole without division.

10. Two kinds of secondary spermatocytes are formed: one with and the other without the X chromo-

some. Hence the first meiotic division is reductional for the X-chromosome in all three genera.

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# Analysis of the Genetical Differentiation in the Species of *Drosophila pseudo-obscura*

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## Introduction

The two races ( A and B ) of Drosophila pseudo-obscura discovered by Lancefield ( I929 ) have been studied genetically as well as cytologically by Koller ( I932, I934, I936 ), Dobzhansky and Boche ( I933 ), Tan ( I935 ), and Dobzhansky ( I934, I935a , I935b, I935c, I936 ). It has been found that while the $F_I$ male hybrids are completely sterile and show differences in testis size in the reciprocal crosses, the $F_I$ female hybrids are partially fertile and exhibit reduction of crossing-over in several chromosomes. This reduction of crossing-over has been shown to be due to the presence of inverted segments within homologous chromosome pairs ( Tan, I935; Koller, I936 ). The species of Drosophila pseudo-obscura is thus differentiated genetically into two partially inter-sterile races,-a differentiation which may possess great evolutionary significance. External differences, such as might serve to distinguish the two races phenotypically, have not been found. The two races as well as the several geographical lines within each race may be regarded as incipient species, species in statu nascendi. A study of their genetical and perhaps physiological differences and of the behaviour of these differences in crosses will throw light on

the particular processes involved in species-differentiation.

The first object of the present study is an analysis of the testis size of adult males in inter- and intra-racial crosses. This morphological character is influenced by genetic factors which show various degrees of differentiation in the two races and different geographical lines. Furthermore, it is our purpose to study by means of the salivary gland chromosomes the mechanism and the rate of the process by which structural heterozygosity is reduced in successive generations.

## Material and Method

The following races and geographical lines were used:

| Race A: | | Race B: | |
|---|---|---|---|
| | I. Cedar City | | 8. Cambel River-4 |
| | 2. La Grande-2 | | 9. Sequoia-8 |
| | 3. Nakusp-7 | | IO. Santa Lucia |
| | 4. Merritt-2 | | II. Seattle-4 |
| | 5. P sp | | I2. Seattle-6 |
| | 6. y sn v | | I3. sn bu |
| | 7. b dy se sp | | |

The stocks No. I to 4 and 8 to I2 were obtained from Prof. Th. Dobzhansky, California Institute of Technology, Pasadena, in I934, and have since been kept inbred in the Institute of Animal Genetics, Edinburgh. The others stocks were placed at my disposal by Prof. F. A. E. Crew. The chromosome constitution of the two races with special reference to the structure of the Y chromosome has been investigated by Dobzhansky ( I935a ), who demonstrated that there are six different types of Y chromosomes. During the present study, it was found that various stocks also differ in fertility in inter- and intra-racial crosses.

Crosses were made by putting two or three pairs of flies in each vial, the food being the raisin food made according to the formula of Offermann and Schmidt in Drosophila Information Service No. 3, I935.

Since testis size is influenced by temperature ( Baker, I934 ), the cultures were kept constant at 24° C. The measurement of the testis was taken within twenty-four hours of the emergence of the male. The testes were dissected out in Ringer, and measured by means of an eyepiece micrometer. Two dimensional measurements were taken, viz. length from apex to base, and breadth representing the largest diameter. For the cytological examination of the salivary gland chromosomes, Painter's technique was employed.

# PART I. TESTIS SIZE

## Testis Size of Different Races and Geographical Lines

Of the different parental lines of race A used during the study, La Grande-2 was collected from Washington, Nakusp-7 and Merritt-2 from British Columbia, and Cedar City from Utah ( Dobzhznsky, I935 ). The mutant stock y sn v carries yellow body-colour, singed bristles, and vermilion eye-colour; P sp denotes Pointed wing-shape ( dominant ), snapt or broken second wing-vein; while b dy se sp stands for beaded wing-edge, dusky wing, sepia eye-colour, and snapt. All these characters are sex-linked mutants of race A. Of the lines of race B, Cambel River-4 was collected from Vancouver Island, Sequoia-8 and Santa Lucia from California, and Seattle-4 and Seattle-6 from Washington. The mutant stock sn bu of race B carries the sex-linked characters of singed bristles and bubble wing. These stocks were chosen because they represent a group with a wide geographical distribution, and hence were expected to show definite and detectable genetic differentiation. The mutations were discovered in inbred populations ( Crew and Lamy, I9 35 ). Sex-linked mutants were specially chosen in order to analyse the relationship between the genic make-up of the sex chromosome and the infecundity of

Table I

Testis size of parental stocks

| Race A: | Length | $\sigma$ | Breadth | $\sigma$ | n |
|---|---|---|---|---|---|
| Nakusp-7 | 648±2.0 | 3I.9 | 264±I.3 | 2I.4 | II7 |
| La Grande-2 | 656±3.I | 46.4 | 279±2.5 | 37.3 | I03 |
| Cedar City | 659±3.3 | 5I.8 | 242±I.5 | 24.0 | II2 |
| y sn v | 663±5.7 | 84.3 | 252±I.8 | 26.2 | 98 |
| Merritt-2 | 703±2.9 | 45.9 | 256±0.8 | I4.I | II7 |
| P sp | 726±4.0 | 59.5 | 298±2.2 | 32.5 | I03 |
| b dy se sp | 754±3.6 | 55.2 | 268±2.4 | 36.5 | I07 |
| Race B: | | | | | |
| sn bu | 533±4.8 | 74.6 | 200±I.6 | 24.7 | I08 |
| Cambel River-4 | 602±4.2 | 55.7 | 204±I.7 | 24.4 | 82 |
| Sequoia-8 | 623±4.3 | 65.2 | 232±I.7 | 26.2 | I08 |
| Santa Lucia | 659±3.3 | 47.5 | 228±I.2 | I7.7 | 94 |
| Seattle-4 | 669±3.3 | 45.5 | 257±I.6 | 22.6 | 87 |
| Seattle-6 | 689±3.0 | 48.9 | 243±I.4 | 24.0 | I20 |

( In the table, $\sigma$ represents the standard deviation; n, the number of testes measured. )

the hybrids as expressed by the size of the testis.

The mean values in micra for the length and breadth of the testis, together with their standard deviation and probable error, have been calculated and are given in table I. It will be noticed that within each race there is considerable variation, Nakusp-7 having the shortest and b dy se sp the longest testis in race A. . Similarly, in race B, the scale ascends from sn bu to Seattle-6. Breadth is Directly proportional to length so that a longer testis would also be broader, but no strict proportionality was observed. In general, flies of race A have a somewhat larger testis than flies of race B, as was first noted by Koller ( I934 ), while mutant stocks show a wider range of variation than normal wild-type strains, as indicated by the slightly larger values for the standard deviation.

## Testis Size of Intra- and Inter-racial Hybrids in Successive Backcrosses

Males of one type only were used, namely, those of Cedar City, race A. In intra-racial crosses, they were mated to the females of three other lines, namely, La Grande-2, Nakusp-7, and Merritt-2, all of the same race, and in inter-racial crosses to females of Santa Lucia, Seattle-4 and Seattle-6, of race B. In both cases, the $F_I$ females were backcrossed to Cedar City males, and this was repeated for three generations. This method of crossing ensured that the cytoplasm theoretically should remain of the same origin and thus enable us to study the effect of racial differentiation in the chromosomes on the cytoplasm itself. The mating system with La Grande-2, for instance, would be as follows:

La Grande-2♀ X Cedar City♂
$F_I$♀ X Cedar City♂
ǂ$F_2$♀ X Cedar City♂
$F_3$♀ X Cedar City♂

From each generation, the testes of about fifty males were dissected out and measured. The data for the intra-racial crosses are summarized in table II.

On comparing this table with table I, paying particular attention to the testis size of the parental

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ǂ$F_2$, not the ordinary $F_2$, but as used in the text, will be understood to denote the offspring of the $F_I$ backcross, and so on with $F_3$ and $F_4$.

Table II

Cedar City♂ crossed with

| Race A♀ | Generation | Length | σ | Breadth | σ | n |
|---|---|---|---|---|---|---|
| La Grande-2 | $F_I$ | 688±2.6 | 39.I | 286±2.5 | 36.7 | 99 |
| | $F_2$ | 7I5±4.2 | 64.3 | 273±2.2 | 34.6 | I05 |
| | $F_3$ | 7I0±2.9 | 44.0 | 28I±2.6 | 38.2 | I02 |
| | $F_4$ | 724±5.6 | 84.2 | 260±I.9 | 28.8 | I02 |
| Nakusp-7 | $F_I$ | 708±2.7 | 42.2 | 322±3.8 | 58.9 | I05 |
| | $F_2$ | 748±3.7 | 56.9 | 275±2.5 | 36.7 | I03 |
| | $F_3$ | 757±3.4 | 53.4 | 3I5±3.3 | 50.5 | I05 |
| | $F_4$ | 7I6±4.4 | 69.5 | 267±2.0 | 3I.2 | I08 |
| Merritt-2 | $F_I$ | 769±3.I | 49.0 | 33I±3.8 | 59.3 | III |
| | $F_2$ | 766±3.9 | 55.8 | 293±2.I | 30.9 | 97 |
| | $F_3$ | 747±3.7 | 54.7 | 297±I.8 | 26.4 | I02 |
| | $F_4$ | 682±4.7 | 70.0 | 272±2.3 | 34.3 | I00 |

Table III

Cedar City♂ crossed with

| Race B♀ | Generation | Length | $\sigma$ | Breadth | $\sigma$ | n |
|---|---|---|---|---|---|---|
| Seattle-4 | $F_I$ | 434±3.5 | 58.9 | 195±I.6 | 25.8 | I08 |
| | $F_2$ | 538±II.I | I53.8 | 2I3±3.9 | 53.I | 86 |
| | $F_3$ | 626±7.I | I05.5 | 249±2.6 | 39.I | I0I |
| | $F_4$ | 646±6.9 | I08.3 | 246±2.3 | 34.I | I04 |
| Seattle-6 | $F_I$ | 445±4.4 | 70.5 | 207±I.8 | 28.2 | II4 |
| | $F_2$ | 543±2.6 | 40.2 | 2I6±3.3 | 50.2 | I05 |
| | $F_3$ | 665±6.0 | 90.6 | 277±2.I | 30.4 | I02 |
| | $F_4$ | 674±4.0 | 62.7 | 263±2.I | 32.I | I08 |

stocks, it is found that in all three crosses there is an increase of a varying degree in the testis size of the $F_I$ hybrids. In crosses with both La Grande-2 and Nakusp-7, the increase is maintained throughout four generations, but while in the first cross the fourth generation actually has the greatest increase, in the second it shows a significant drop. The third case, Merritt-2, is somewhat different; testis size decreases with further backcrossing, the decrease being first slow, then more rapid. The values for the standard deviation indicate a wider range of variation in testis size in the later generations.

The data for the inter-racial crosses are summarized in table III. In crosses of B♀ X A♂, it was found by Lancefield ( I929 ) that testis size is reduced. Our data show that after repeated backcrossing testis size continuously increases, so that in $F_4$ it is very nearly the 'normal' found in the paternal line. The data obtained with Seattle-4 and Seattle-6 are very similar, except that in the cross with Seattle-4 the range of variation, as indicated by $\mu$, is greater in $F_2$, $F_3$, and $F_4$. The cross with Santa Lucia failed in spite of persistent trials.

The different testis size of intra- and inter-

racial hybrids in successive generations calls for explanation. By repeated backcrossing the chromosome complement in both crosses comes to be made up partially or completely of chromosomes introduced only from the male side. If the increase of testis size in the $F_I$ of intra-racial crosses and the great decrease in the $F_I$ of inter-racial crosses are due to an interaction between foreign chromosomes, i.e. chromosomes given by the male parent, and the egg cytoplasm, we should then expect a further and continuous increase or decrease in testis size in the following generations. The data obtained in the crosses with La Grande-2 and Nakusp-7 support this assumption, but other facts, such as decrease of testis size in Merritt-2 cross and its increase in inter-racial crosses, render this explanation insufficient.

If the increase or decrease of testis size is brought about by a simple interaction between the hybrid chromosome sets only, it should be expected that after repeated backcrossing the size of testis would be the same as in the males of the original paternal line, e.g. testis size of $F_4$ males of the cross between Merritt-2 and Cedar City should be around the value of 659 $\mu$, which is the length of testis in Cedar City stock. The data given in

table III, however, show that this is not so.

Two interesting facts may be noted: Firstly, the testis size of $F_4$ hybrids in different intra-racial crosses, where the homozygosity of chromosomes in the complement by backcrossing is probably nearly attained, is not consistent but shows various degrees of deviation from that found in the paternal line, e.g. Merritt-2♀ X Cedar City♂ $F_4$ males have a testis length of 682$\mu$, which is nearly the mean value for testis size in the two parental lines. In the other crosses, e.g. in La Grande-2♀ X Cedar City♂ and Nakusp-7♀ X Cedar City♂, the deviation is found to be even greater.

Secondly, testis size in successive hybrid generations of the same cross exhibits a considerable and significant variation either in the positive or negative direction: that is, the testis of $F_I$ males may be larger than the testis of $F_4$ males of the same cross, e.g. in Merritt-2; or smaller, e.g. in La Grande-2 and Nakusp-7.

These facts strongly suggest that testis size is not determined by a simple interaction either between the foreign chromosomes and egg cytoplasm or between the hybrid chromosome sets, but the process is more complex and probably involves factors which are specific to the different endogamous groups and

complementary in their functions during development.

## Testis Size of Inter-racial Hybrids in the Presence of Mutant Genes

The effect of different constitutions of the sex chromosome on testis size was studied by making reciprocal crosses of sex-linked mutant stocks with normal wild-type strains. Since the sex-linked mutant genes tested all belong to race A, they should be present in the male offspring of the cross A♀ X B♂ but not in the offspring of the cross B♀ X A♂. The offspring of the reciprocal crosses should, therefore, show differences in testis size, if any, owing to the presence or absence of these factors, apart from differences due to hybridity ( decrease in the B♀ X A♂ ). Stocks having two, three, and four sex-linked mutant genes were tested. The data for two genes are summarized in table IV.

In the A♀ X B♂ cross, there is no appreciable change in testis size of $F_I$ hybrids as compared with that of parental races and especially the paternal race, e.g. in P sp testis length is 726μ, in Cambel River-4 602μ, and in $F_I$ hybrids 631μ, which is between the two values but nearer to that of Cambel River-4, which was the male parent. The only exception was found in the cross with Santa Lucia, where there is a considerable decrease. In the B♀ X A♂ cross, there is great decrease in testis size

Table IV

| A/B | Length | $\sigma$ | Breadth | $\sigma$ | n |
|---|---|---|---|---|---|
| P sp♀ X Santa Lucia♂ | 591±3.1 | 47.4 | 273±2.3 | 35.6 | 105 |
| P sp♀ X sn bu♂ | 603±8.8 | 118.6 | 259±2.1 | 28.2 | 83 |
| P sp♀ X Sequoia-8♂ | 620±3.4 | 51.9 | 264±1.5 | 23.7 | 107 |
| P sp♀ X Cambel River-4♂ | 631±2.7 | 43.0 | 294±2.0 | 31.1 | 113 |
| B/A | | | | | |
| Santa Lucia♀ X P sp♂ | 352±3.0 | 38.5 | 199±2.1 | 26.7 | 77 |
| sn bu♀ X P sp♂ | 396±4.5 | 57.8 | 185±2.8 | 35.6 | 76 |
| Sequoia-8♀ X P sp♂ | 556±5.9 | 32.6 | 251±1.9 | 10.4 | 14 |
| Cambel River-4♀ X P sp♂ | 485±4.4 | 65.3 | 202±1.8 | 26.7 | 102 |

except in the hybrid males of the cross Sequoia-8♀ X P sp♂, where the decrease is slight. The data suggest that the effect of sex-linked mutant genes on testis size, if any, is negligible and inconsistent. It should be noted that in the cross mutant X mutant ( sn bu X P sp ) the standard deviation is great, indicating a wide range of variation.

The results for three sex-linked mutant genes are summarized in table V. In these crosses, even in the A♀ X B♂, there is a slight general decrease in testis size. In the reciprocal cross, B♀ X A♂, as expected, the decrease is much greater. It is interesting to note that two crosses, sn bu♀ X y sn v♂ and Santa Lucia♀ X y sn v♂, failed notwithstanding persistent efforts while the reciprocal crosses were successful. In the B♀ X A♂ cross , the results were similar to those involving two sex-linked mutant genes; the decrease is slight in the hybrid males of the cross with Sequoia-8.

The data for four sex-linked mutant genes are summarized in table VI. They show that in the A♀ X B♂ cross there is no change in testis size of the hybrid males. The reciprocal cross results in the usual great decrease of testis size. Again, the decrease is smaller in the cross with Sequoia-

Table V

| A/B | Length | $\sigma$ | Breadth | $\sigma$ | n |
|---|---|---|---|---|---|
| ysnv♀ X Sequoia-8♂ | 529±3.9 | 57.8 | 249±2.I | 3I.I | IOI |
| ysnv♀ X Cambel River-4♂ | 538±3.4 | 50.4 | 22I±I.7 | 25.2 | I02 |
| ysnv♀ X sn bu♂ | 539±4.9 | 78.6 | 2I2±I.9 | 3I.I | II8 |
| ysnv♀ X Santa Lucia♂ | 548±3.5 | 53.4 | 247±I.3 | I9.3 | I03 |
| B/A | | | | | |
| Sequoia-8♀ X ysnv♂ | 425±4.0 | 47.4 | 223±I.9 | 22.2 | 62 |
| Cambel River-4♀ X ysnv♂ | 337±4.I | 62.3 | I69±I.3 | I9.3 | I05 |
| sn bu♀ X ysnv♂ | failed | | ---------- | | |
| Santa Lucia♀ X ysnv♂ | failed | | ---------- | | |

Table VI

| A/B | Length | σ | Breadth | σ | n |
|---|---|---|---|---|---|
| bdysespo♀ X Sequoia-8♂ | 631±3.0 | 45.9 | 318±2.1 | 31.1 | 104 |
| bdysespo♀ X sn bu♂ | 636±5.7 | 77.1 | 313±3.8 | 51.9 | 84 |
| bdysespo♀ X Santa Lucia♂ | 648±4.2 | 48.9 | 271±2.9 | 34.1 | 61 |
| bdysespo♀ X Cambel River-4♂ | 658±5.8 | 51.9 | 251±2.6 | 23.7 | 37 |
| B/A | | | | | |
| Sequoia-8♀ X bdysespo♂ | 519±3.0 | 45.9 | 254±2.1 | 32.6 | 109 |
| sn bu♀ X bdysespo♂ | 388±3.6 | 57.8 | 197±2.0 | 32.6 | 119 |
| Santa Lucia♀ X bdysespo♂ | 378±3.1 | 56.3 | 214±1.7 | 31.1 | 146 |
| Cambel River-4♀ X bdysespo♂ | 470±1.2 | 19.3 | 235±1.6 | 25.2 | 114 |

8 and the standard deviation for mutant X mutant is greater than for mutant X wild-type. The fact that in different crosses of stock sn bu, race B, the standard deviation is consistently high, suggests that the presence of these genes decreases the uniformity of testis size not only in hybrids but even in the parental pure stock.

The data given in tables IV, V, and VI show that apart from differences due to hybridity, the reciprocal crosses show little differences in testis size due to the presence or absence of sex-linked mutant factors. Furthermore, where in the A♀ X B♂ cross a slight decrease is found, the effect is not in general additive. Rather, a qualitative difference exists, so that some mutant gene combinations exert an effect on testis size, e.g. P sp and y sn v, while others, though they involve a greater number of genes, e.g. b dy se sp, do not have any such effect. This suggests that the genom of the mutants and the various races is qualitatively differentiated as regards its influence during development through the presence of particular genetic factors.

## Discussion

The data described in the preceding sections indicate that the increase or decrease of testis size in successive hybrid generations of the various crosses cannot be adequately explained by a simple interaction between foreign chromosomes and the egg cytoplasm, or an interaction between hybrid chromosomes alone. Our data suggest that testis size in the pure races and lines, though dependent primarily upon genetic factors, is determined by a complex interaction between these factors, which are complementary, and the cytoplasm. The following hypothesis is offered. Both the genes and the cytoplasm undergo differentiation, which may be causally correlated,— cytoplasmic differentiation being conditioned by genic differentiation. In the races and geographical lines, such combined differentiation has brought about the differences in testis size, the degree of difference depending upon the degree of differentiation. Our data suggest that in the hybrids the cytoplasm exerts a varying degree of influence on testis size by modifying the direct effect of the interaction between chromosome complements containing the complementary genetic factors. In the hybrids where through repeated backcrossing the testis size gradually reverts back to

normal, e.g. Cedar City♂ with Merritt-2♀, Seattle-4♀, and Seattle-6♀, it is probable that the divergence in differentiation with regards to the complementary genetic factors and cytoplasm is small, and in the third or fourth hybrid generation when the chromosome constitution of the hybrids is largely of Cedar City, the cytoplasm ( from Merritt-2 , Seattle-4, and Seattle-6 ) can apparently affect the testis size characteristic of Cedar City but little. In the other crosses, where an increase in testis size is maintained in the successive generations ( Cedar City♂ with La Grande-2♀ and Nakusp-7♀ ), the genetic factors and the cytoplasm of the females are characterized by a greater differentiation; the degree of interaction will be great; consequently, the larger testis size of $F_3$ and $F_4$ hybrids will be maintained and even increased. If this hypothesis is correct, we should be able to classify the various geographical lines of Drosophila pseudo-obscura on the basis of the increase or decrease in testis size of successive generations when backcrossed to a single standard line and thus determine the degree of differentiation their complementary genetic factors and cytoplasms have undergone relatively to one another. On this basis, we would deduce that the genetic systems, including

both the chromosomes and the cytoplasm, of Seattle-4, Seattle-6, and Merritt-2 are more similar to that of Cedar City than those of La Grande-2 and Nakusp-7.

Certain geographical lines have in fact been classified by Dobzhansky and Boche ( I933 ) as"physiologically strong" or "physiologically weak", on a basis essentially similar to the above but limited to the testis size of the first hybrid generation. Accordingly, a race B which when crossed to A gives hybrids with very small testes may be called a strong B race; while one giving relatively large testes, a weak race. Analysing tables IV , V, and VI from this point of view, it is seen that the different geographical lines can be consistently arranged into the following series: Santa Lucia , sn bu, Cambel River-4, Sequoia-8, according to the degree of their differentiation. The stocks Seattle-4 and Seattle-6 may be inserted between sn bu and Cambel River-4 according to data in tables IV and VI. We note that sn bu in its crosses with various race A lines gives a high standard deviation, and that Seattle-4 gives a higher deviation in its cross with Cedar City than Seattle-6. Both these lines have been classified as physiologically strong ( Koller, I936 ), and one may assume

from such behaviour of the strong races that the greater degree of differentiation is connected with a greater degree of variation.

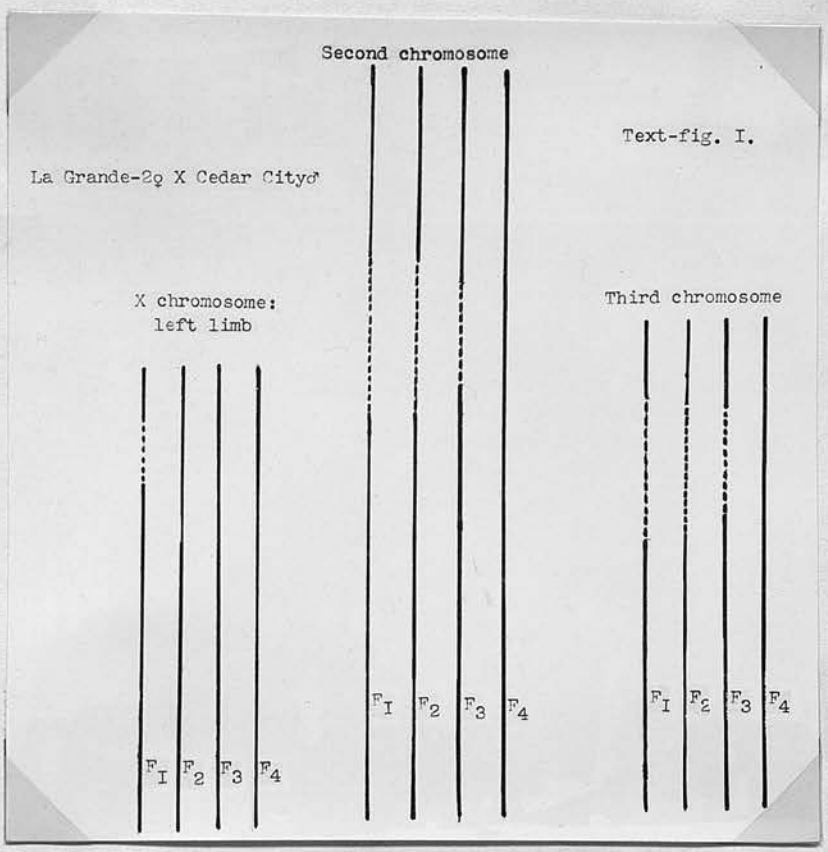
## PART II. STRUCTURAL HYBRIDITY

### The Structure of Salivary Gland Chromosomes of Intra- and Inter-racial Hybrids in Successive Backcrosses

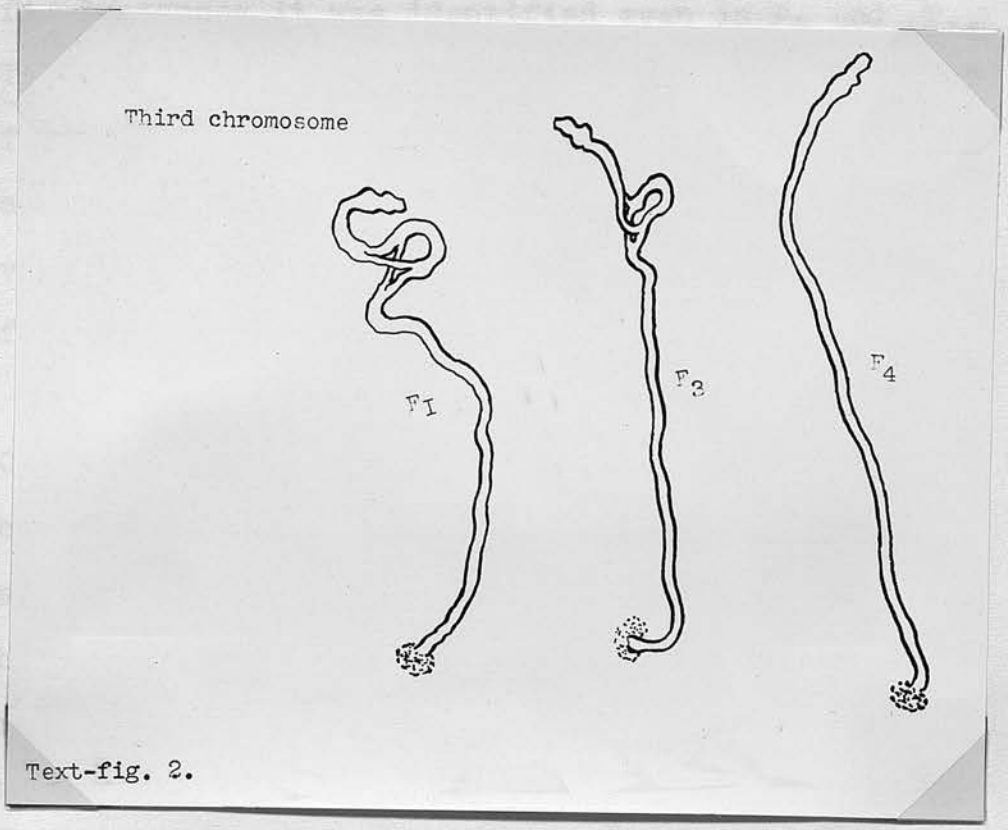
The chromosomes of the salivary glands were examined with a view to drawing some inference regarding chromosome behaviour in the successive hybrid generations. The hybrids investigated belonged to the crosses of Cedar City with various race A and race B lines.

Some $F_I$ individuals in the intra-racial cross La Grande-2♀ X Cedar City♂ showed one or more of three different inversions: one in the left limb of the X chromosome, rather small and near the end; another in the second chromosome , of considerable length, also near one end but extending to the middle; and a third short one in the third chromosome also near one end. In $F_2$, the inversion in the X chromosome was not detected, the other two inversions remained almost the same as regards length. Both these inversions were found in $F_3$, though considerably reduced in extent, and were not found in $F_4$. Diagrammatically, the chromosome constitution in the successive generations is shown in figs. I and 2.

In the $F_I$ of the intra-racial cross Nakusp-7♀ X Cedar City♂, only two inversions were found: one in




Text-fig. I. Diagram illustrating the chromosome constitution in successive generations of back-crosses in the intra-racial cross La Grande-2♀ X Cedar City♂.



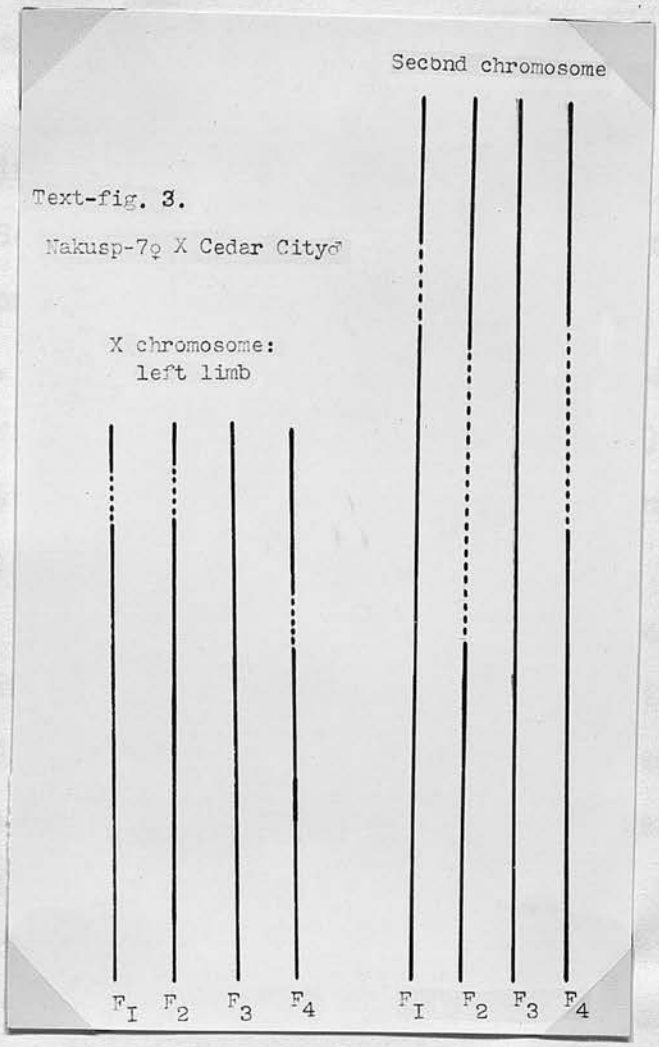

Text-fig. 2.

Text-fig. 2. Drawings of the third chromosome in the salivary gland nuclei of La Grande-2♀ X Cedar City♂ showing the decrease of inversion in successive generations.

the left limb of the X chromosome, very small, and the other in the second chromosome and also small. In $F_2$, the inversion in the left limb of the X chromosome had disappeared in most cases, but in a few instances it was identified even in $F_3$ and $F_4$. The inversion in the second chromosome was identified in $F_2$ and $F_4$, but the one in $F_2$ was larger than that in $F_I$, and might well be a newly arisen inversion. The chromosome constitution is shown in fig. 3.

The third intra-racial cross Merritt-2♀ X Cedar City♂ yielded results very similar to those in the cross with La Grande-2. It gave three inversions, apparently the same as those in the latter cross , because they occupied the same positions in the chromosomes. However, they persisted longer, and were found, though considerably reduced,in $F_2$ and $F_3$, and completely disappeared only in $F_4$.

These crosses show that even within the same race ( race A ), differences in gene sequence are present which may be considered as characteristic of the different geographical lines. This finding agrees closely with the report by Koller of the spontaneously arising structural changes in the chromosome complement of Drosophila pseudo-obscura ( I9 36 ). Similar observation was made by Darlington

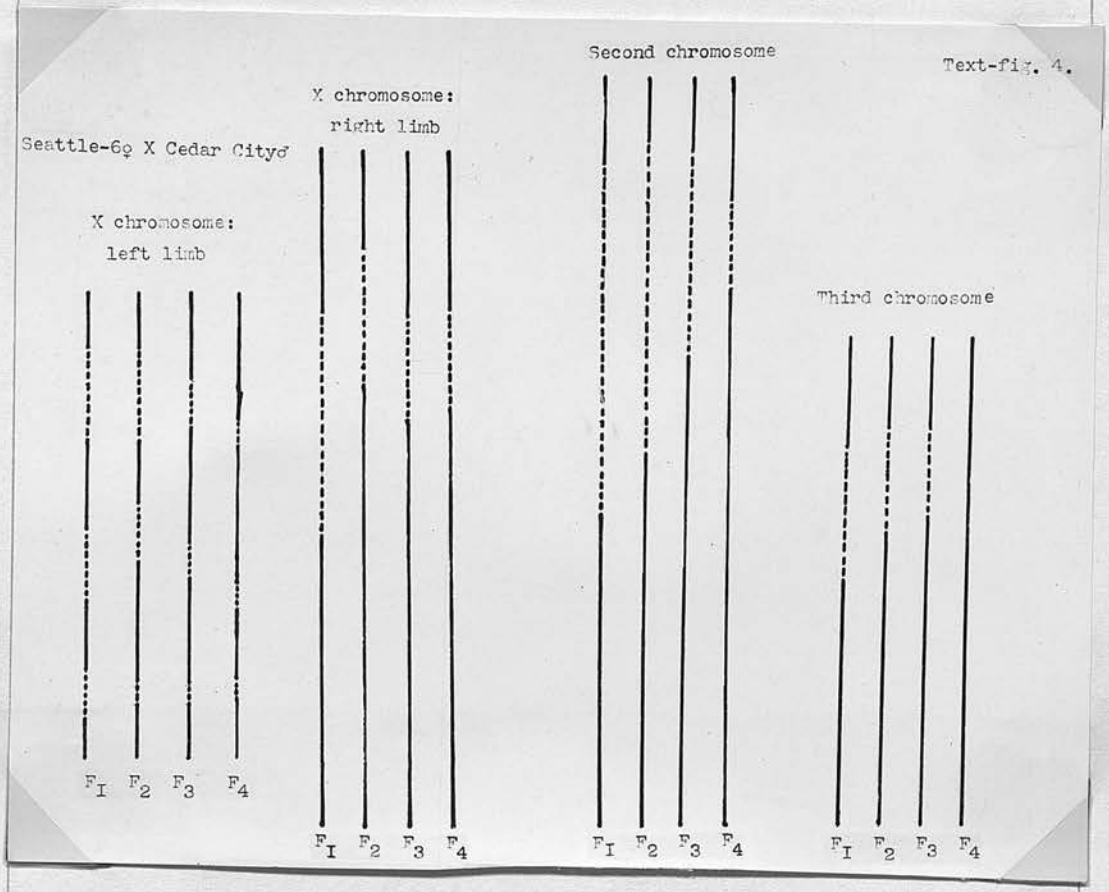



Text-fig. 3. Diagram illustrating the chromosome constitution in successive generations of back-crosses in the intra-racial cross Nakusp-7♀ X Cedar City♂.

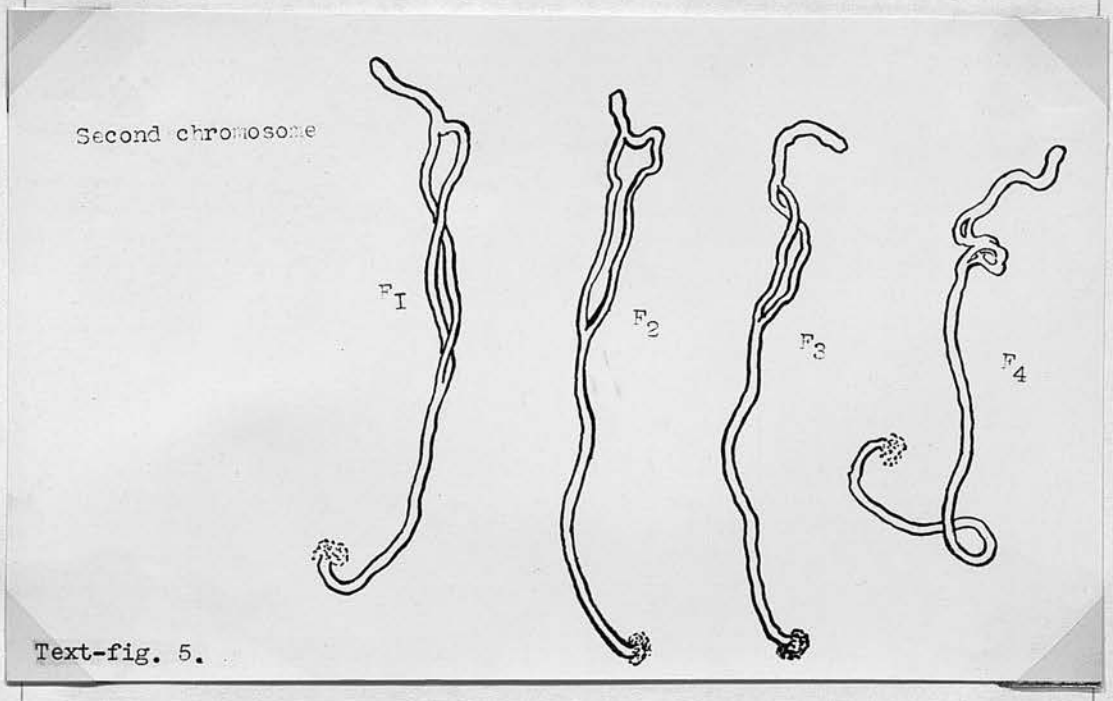
on grasshoppers ( I936 ). These facts and our own data strongly suggest that structural changes in the chromosome complement of individuals within a population are of wide-spread occurrence.

In the inter-racial crosses Seattle-4♀ X Cedar City♂ and Seattle-6♀ X Cedar City♂, similar structural differences in the chromosome complement were identified. Six inversions were present; they were identical with those reported by Tan ( I935 ): three in the left limb of the X chromosome, one in the right limb; one in the second chromosome; and one in the third. In the backcrosses, these inversions showed great variation in number and length: in some larvae of $F_2$ one or more of the inverted segments were decreased in length, in others some inversions were entirely absent. Generally, however, they were present in $F_2$, $F_3$, and $F_4$, but were shorter than in $F_I$. The inversion in the third chromosome disappeared altogether in $F_4$. The chromosome structure of the hybrids is shown in figs. 4 and 5.

The analysis of the salivary gland chromosomes in the intra- and inter-racial hybrids in successive generations when the backcrossing was made to the same type of male ( in our case Cedar City ) thus shows that the hybrids soon attain structural homozygosity in the chromosome complement. This homozygosity is due primarily to two phenomena ,




Text-fig. 4. Diagram illustrating the chromosome constitution in successive generations of back-crosses in the inter-racial cross Seattle-6♀ X Cedar City♂.




Text-fig. 5.

Text-fig. 5. Drawings of the second chromosome in the salivary gland nuclei of Seattle-6♀ X Cedar City♂ showing the decrease of inversion in successive generations.

crossing-over within the inverted segments, and chromosome segregation. Crossing-over occurs frequently within the inverted segments of homologous chromosomes, as was demonstrated genetically by Sturtevant and Beadle ( I936 ). Cytological investigations show that within an inversion both single and double crossing-over takes place( Richardson, I935 ). Single crossing-over results in the production of two normal chromatids, one dicentric chromatid, and one acentric fragment. The latter element is eliminated during the first anaphase. The dicentric chromatid breaks either at the first or at the second meiotic anaphase, and gametes receiving the deficient chromosomes will be inviable. The lower fecundity of the hybrid females found in the interracial crosses is thus due to crossing-over within inverted segments. Single crossing-over,therefore, should be looked upon as a process by which structural heterozygosity, brought about by intercrossing of the endogamous groups, will be reduced and ultimately eliminated, specially if the structural change is not associated with further genic differentiation which would make intercrossing a principio ineffective. It may be seen that the elimination of inverted segments in the chromosome complement of the hybrid La Grande-2♀ X Cedar City♂ is a fairly

rapid process.

The interesting observation made in the cross Nakusp-7♀ X Cedar City♂, namely, that in $F_2$ the inversion in the second chromosome is as much as four times larger than the same inversion in $F_I$, indicates that this inversion may have originated spontaneously and independently of the inter-racial inversions of the $F_I$ hybrid. This paricular inversion in $F_2$ occupies a much more median position than the inter-racial inversion in $F_I$, and furthermore, does not overlap the position of the latter. It seems reasonable to assume, therefore, that Nakusp-7♀ X Cedar City♂ hybrids in the experiment underwent a structural change, either spontaneous or conditioned by hybridity, and females selected for backcrossing carried the new inversion.

Another interesting fact was that in the inter-racial $F_2$, $F_3$, and $F_4$ hybrids, the length of inversions apparently decreased though the number of inversions remained the same as in the $F_I$. This fact suggests that besides single crossing-over within inverted segments, there is a more or less frequent reinversion. Our observation suggests that in some cases only a very small region of the original inversion was left over, which could not be detected cytologically because it involved only a few genes.

The other fact assumed to be responsible for the elimination of inversions is chromosome segregation. Since this is at random, it will necessarily result in gametes which contain chromosomes of the same origin. It is probable that individuals derived from the fertilization of an ovum by a sperm having chromosomes of the same origin will be more viable than individuals who carry chromosomes of two races in their chromosome complement. It is very probable that during the study of the salivary gland chromosomes, the great number of $F_2$, $F_3$, and $F_4$ larvae were more or less selected for their vigour and healthier condition, which may be taken as a proof that they possessed a more or less homozygous chromosome complement.

The facts discussed above are of special interest from an evolutionary point of view. The structural changes in the chromosome complement within a species will change independent gene inheritance to gene-group inheritance by reducing crossing-over between linked genes, which, as a reault, reduces variability within the species( Darlington, I936 ). The increase in linkage intensity of certain genes localized in the region involved in the structural change may be of adaptative value, but this is only temporary and is counterbalanced by the great disad-

vantage of reducing variability. The single crossing-over within inverted segments provides a mechanism which leads to partial or complete elimination of the inversion. Hence it may be considered to be primarily responsible for the maintenance of structural homozygosity of endogamous groups within a species and in a way prevents the mixing of these groups. In Drosophila pseudo-obscura, the structural differences are not responsible for the origin of these groups; they are important only in maintaining their isolation. We have seen that in the hybrids of these endogamous groups the structural differences in the chromosome complement can be eliminated; this proves that they are of secondary importance to the genic differentiation, which alone is responsible for the divergence of species into different races.

Comparisons of testis size and the number and length of inversions in hybrids of successive backcross generations show that the two phenomena are not causally related. In the intra-racial cross La Grande-2♀ X Cedar City♂, the inversions were rapidly eliminated so that in $F_4$ no inversion was found. The increase in testis size, however, persisted, and was even slightly greater in $F_4$ than in the earlier generations. Similarly, in the inter-racial cross Se-

attle-6♀ X Cedar City♂, most of the inversions were still found in $F_4$, although considerably reduced in size. The testis size, however, had already reverted back to normal, the large decrease having entirely disappeared. This further corroborates the conclusion of Dobzhansky ( I936 ), that inversions in the inter-racial hybrids are not the cause of male sterility.

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## Summary


I. Race A males of Drosophila pseudo-obscura were found to possess larger testes than males of race B; the difference is statistically significant.

2. Mutant stocks of Drosophila pseudo-obscura were found to show a slightly greater variation in testis size than normal wild-type strains. In inter-racial hybrids, "strong" races show greater variation than"weak" races.

3. Testis size shows an increase in intra-racial hybrids and a decrease in inter-racial hybrids as compared with that in parental stocks. The increase in the intra-racial hybrids is maintained in successive generations of backcrossing in certain crosses, but disappears in other crosses. The decrease in $F_I$ inter-racial hybrids is great in the B♀ X A♂ cross, but slight and sometimes absent in the reciprocal cross. This decrease disappears with repeated backcrossing.

4. A complex interaction between complementary genetic factors in the hybrid chromosomes and the cytoplasm is assumed to be responsible for the apparently inconsistent behaviour of testis size in the various crosses. The modifying effect of the cytoplasm is assumed to be due to cyto-

plasmic differentiation conditioned by genic differentiation in the genetic system of the different geographical lines.

5. Sex-linked mutations have no general additive effect on testis size. Particular sex-linked mutant genes, however, do exert some effect on testis size, the data suggesting that the qualitative differentiation of the gene-complex as expressed by the presence of these genes may have an influence.
6. Two or three inversions were found in the chromosome complement of some intra-racial hybrids and six inversions in the chromosome complement of the inter-racial hybrids.
7. The intra-racial inversions were gradually eliminated in successive generations of backcrosses; the rate of elimination being greater than in the inter-racial hybrids. In the latter, the length rather than the number of inversions was reduced.
8. The elimination of inversions is ascribed to crossing-over within the inversions and chromosome segregation. Both single and double crossing-over are assumed to occur.
9. Crossing-over within inverted segments of chromosomes is considered not only to be responsible

for maintaining structural homozygosity in the chromosome complement of the endogamous groups within a species, but to prevent the successful intermixing of the endogamous groups and hence lead to further differentiation of these groups.

IO. Comparisons of testis size behaviour and of the behaviour of structural changes in the chromosome complement show that the latter have no causal connection with the phenomenon of hybrid sterility in the male hybrids.

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# Six New Mutations and their Linkage Relationship in *Drosophila pseudo-obscura*

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## Introduction

Among the several species of Drosophila which are under extensive genetical analysis, Drosophila pseudo-obscura has the advantage of possessing intersterile geographical races, and hence offers material not only for genetical investigations but for the study of species from the point of view of their evolution. Studies in this last respect, however, are much hampered by the fact that in D. pseudo-obscura, relatively few genes are known in the various linkage groups. The lack of sufficient markers in the chromosomes makes it difficult to undertake a detailed comparison of the chromosomes in various geographical races and lines concerning their gene arrangement. It is therefore desirable to obtain more genetical data for the construction of chromosome maps, so that it may be possible to study those genetical and structural changes which take place within the species and lead to the isolation of endogamous groups.

In 1936, several new mutations were discovered mostly in X-rayed stock of D. pseudo-obscura, race A, by Miss Rowena Lamy, and they were reported in D. I. S. No. 7, 1937. These are distributed in four chromosomes and their positions make them valuable markers for genetical analysis. The present

paper describes six of these new mutations in detail and gives the data of genetical analysis which was undertaken in order to determine their loci in the chromosome maps.

## The X Chromosome

### ( 1 ) Blistered ( bli )

This mutation was found as a single male among the progeny of an X-rayed female. It is characterized by a varying degree of crumpling and blistering of the wings which are also often spread. One or both wings may be affected. Flies with this character are unable to fly but are otherwise quite viable.

To test the linkage group of blistered, a blistered female was crossed to a wild-type male. This first cross gave blistered sons and wild-type daughters, showing that blistered is a sex-linked recessive. A subsequent test indicated that blistered is near the middle of the X chromosome. Three known genes were involved in this cross, scutellar ( sc, missing scutellar bristles; locus, 62 ), yellow ( y, yellow body-colour; locus, 62.3 ), and singed ( sn, singed bristles; locus, 70 ). The method of crossing is as follows:

$P_1$ $\frac{sc\ y\ sn}{sc\ y\ sn}$ ♀ X $\frac{bli}{}$ ♂

$F_1$ ♂ $sc\ y\ sn$ $\qquad$ $\frac{sc\ y\ sn\ +}{+\ +\ +\ bli}$ ♀ X $\frac{+\ +\ +\ +}{}$ ♂

$F_2$ ♀ $+\ +\ +\ +$ $\qquad$ ♂ 16 classes

( Classification of $F_2$ males is given in table 1. )

Table 1

Recombination data of males from the cross

$\frac{sc\ y\ sn}{bli}$ ♀ X $\frac{+\ +\ +\ +}{}$ ♂ ( 24 pair matings )

| Types of crossing-over | Locus of c. o. | Classes | No. of ♂♂ | No. of ♀♀ |
|---|---|---|---|---|
| non- | | $\frac{sc\ y\ sn}{bli}$ | 1030 | |
| | | $\frac{}{+\ +\ +\ +}$ | 1107 | |
| single | sc/y | $\frac{sc\ bli}{}$ | 1 | |
| | | $\frac{y\ sn}{}$ | 1 | |
| single | y/sn | $\frac{sc\ y\ bli}{}$ | 112 | |
| | | $\frac{sn}{}$ | 134 | |
| single | sn/bli | $\frac{sc\ y\ sn\ bli}{}$ | 104 | |
| | | $\frac{+\ +\ +\ +}{}$ | 152 | |
| double | sc/y,y/sn | $\frac{sc\ sn}{}$ | 0 | |
| | | $\frac{y\ bli}{}$ | 3 | |
| double | y/sn,sn/bli | $\frac{sc\ y}{}$ | 8 | |
| | | $\frac{sn\ bli}{}$ | 8 | |
| double | sn/bli,sc/y | $\frac{y\ sn\ bli}{}$ | 0 | |
| | | $\frac{sc}{}$ | 0 | |
| triple | sc/y,y/sn, sn/bli | $\frac{y}{}$ | 1 | |
| | | $\frac{sc\ sn\ bli}{}$ | 1 | |
| | | Total | 2649 | 2917 |

Recombination

sc/y, .26%  y/sn, 10.0%

sn/bli, 10.3%  y/bli, 19.4%

From this table, it is computed that blistered is 10.3 units from singed and 19.4 units from yellow . Blistered, therefore, cannot be on the same side of singed as yellow, but it must be on the other side. Singed is located at 70( Donald,, 1936 ), therefore blistered is placed at 80. As a check upon our work , the percentages of recombination between scutellar and yellow and between yellow and singed have been calculated. The distance between scutellar and yellow is .2 unit and that between yellow and singed is 10.0 units. The distance between scutellar and yellow as found by Lancefield ( 1922 ) was .3 unit and that between yellow and singed as found by Donald ( 1936 ) was 8 units. The agreement is fairly close.

Donald ( 1936 ) localized a dominant gene Rounded-nick at 78 in the X chromosome ( Rn, rounded end of wing with a small piece of the wing missing). Since this is close to the position of blistered, and both genes affect the same organ, the wing, to test their actual relationship, a further analysis was undertaken. A blistered female was crossed to a Rounded-nick male. Both the sons and daughters of this cross appeared blistered, while the wings of the daughters also showed signs of Rounded-nick. The expression of blistered in the females indicates that blistered is an allele of Rounded-nick. In order

to verify this assumption, these $F_1$ females were crossed to wild-type males. They produced two kinds of sons, blistered and Rounded-nick, and no recombination was observed. The data thus proved that blistered is an allele of Rounded-nick.

In race B, a sex-linked recessive gene called bubble was described by Lancefield ( 1929 ). Bubble also affects the wings; its effect is to accumulate in some region of the wing a small drop of water which dries off rather slowly during the time of emergence. After the water has dried, the wing becomes impaired in the region which contained the bubble. Blistered appeared to be a more extreme form of bubble. In order to test whether there is any relationship between the two mutations, interracial crosses were made between blistered and bubble. The $F_1$ hybrid females had impaired wings which apparently showed both characters superimposed. This shows that bubble and blistered are alleles.

( 2 ) Erect ( er )

This mutation was also obtained from X-rayed stock. It affects the wings; they are turned upward at a varying angle. The manifestation of this gene is rather variable, and there is some overlapping with wild-type. Egg-laying is often delayed, the time of hatching is consequently three or four days later than in wild-type. Also, a number of eggs laid do not develop, and the adult flies show rather poor viability.

A preliminary cross er♀ X +♂ gave erect sons and wild-type daughters. This showed that erect is a sex-linked recessive. In order to test its position on the X chromosome, it was mated to sepia lanceolate snapt ( se, sepia eye-colour, locus— 1 16 ; ll, lanceolate wing-shape, locus— 140; sp, broken second wing-vein, locus— 157 ). The method of crossing is shown below:

$P_1$ er♀ X se ll sp♂

$F_1$ $\frac{er}{+}$♂ X $\frac{se\ ll\ sp}{er}$♀

$F_2$ $\frac{+\ +\ +\ +}{+}$♀ ♂ 16 classes

( Classification of $F_2$ males is given in table 2.)

Table 2

Recombination data of males from the cross

$\frac{\text{se ll sp}}{\text{er}}$ ♀ X + + + + ♂ ( 5 ♀ pair matings )

| Types of crossing-over | Loci of c. o. | Class | No. of ♂♂ | No. of ♀♀ |
|---|---|---|---|---|
| non- | | se ll sp | 42 | |
| | | er | 36 | |
| single | ll/sp | se ll er | 4 | |
| | | sp | 5 | |
| double | er/se, se/ll | se er | 16 | |
| | | ll sp | 17 | |
| triple | se/ll, ll/sp, er/se | se sp er | 0 | |
| | | ll | 4 | |
| single | er/se | se ll | 10 | |
| | | sp er | 10 | |
| double | er/se, se/ll | ll sp er | 12 | |
| | | se | 29 | |
| double | se/ll, ll/sp | ll er | 4 | |
| | | se sp | 0 | |
| single | sp/er | se ll sp er | 38 | |
| | | + + + + | 53 | |
| | | Total | 286 | 402 |

Recombination

se/er, 47.5%

ll/er, 50.6% ( not allowing for double crossing-over )

sp/er, 54.5% ( not allowing for double crossing-over )

From the above data, it can be seen that the gene erect must be located at the extreme left end of the X chromosome, since it gives nearly 50% of recombination with each of the genes sepia, lanceolate, and snapt. The recombination value is the smallest between er and se, which indicates that the sequence of the genes is: er--se--ll--sp.

To determine the more exact position of erect in the X chromosome, another cross was made, using the genes vermilion ( v, vermilion-coloured eyes; locus, 72 ) and miniature$^2$ ( $m^2$, small wings; locus, 74 ). The results are given in table 3.

Table 3

Recombination data of males from the cross

$\frac{er}{v\ m^2}$♀ X $\frac{+\ +\ +}{}$♂ ( 7 pair matings )

| Types of crossing-over | Loci of c. o. | Class | No. of ♂♂ | No. of ♀♀ |
|---|---|---|---|---|
| non- | | $\frac{v\ m^2}{}$ | 256 | |
| | | $\frac{er}{}$ | 172 | |
| double | er/v, v/$m^2$ | $\frac{v\ er}{}$ | 2 | |
| | | $\frac{}{m^2}$ | 6 | |
| single | v/er | $\frac{m^2\ er}{}$ | 3 | |
| | | $\frac{v}{}$ | 6 | |
| single | $m^2$/er | $\frac{v\ m^2\ er}{}$ | 72 | |
| | | $\frac{+\ +\ +}{}$ | 117 | |
| | | Total | 648 | 714 |

Recombination

v/$m^2$, 2.6%

v/er, 30.4%

$m^2$/er, 30.5% ( not allowing for double crossing-over )

$m^2$/er, 33.0% ( allowing for double crossing-over )

From the above data, erect is seen to give about 33% of recombination with $m^2$. These data, taken in connection with those obtained in the first cross, indicate that the gene-sequence is: er—v—$m^2$. Erect is thus 33 units far on the left side of miniature$^2$. The locus of miniature$^2$ is at 74, therefore erect should be placed at 41. Since the distance is still to large ( between v and er, 30 units ), to show the exact position of erect, a third cross has been made, erect to beaded ( b, beaded wing-edge; locus, 20 ) yellow ( yellow body-colour; locus, 62 ). The results of this cross are given in table 4.

Table 4

Recombination data of males from the cross

$$\frac{b \quad y}{er}\,♀ \times \frac{+\ +\ +}{}\,♂ \quad (\ 12 \text{ pair matings }\ )$$

| Types of crossing-over | Loci of c. o. | Class | No. of ♂♂ | No. of ♀♀ |
|---|---|---|---|---|
| non- | | $\frac{b\ y}{}$ | 274 | |
| | | $\frac{}{er}$ | 244 | |
| double | b/er, er/y | $\frac{b\ er\ y}{}$ | 26 | |
| | | $\frac{+\ +\ +}{}$ | 51 | |
| single | y/er | $\frac{b}{}$ | 108 | |
| | | $\frac{y\ er}{}$ | 60 | |
| single | b/er | $\frac{y}{}$ | 26 | |
| | | $\frac{b\ er}{}$ | 17 | |
| | | Total | 805 | 950 |

Recombination

b/er, 14.9%

y/er, 30.4%

b/y, 45.3% ( allowing for double crossing-over )

The data thus show that the gene erect is localized between beaded and yellow. Its position is 34 on the chromosome map. It is very near to dent. Since between beaded and yellow there was a large gap in the chromosome map and dent is not a good character, erect proves to be a useful mutant in marking an important region of the X chromosome.

( 3 ) Tiny-bristle ( tb )

Tiny-bristle was found as a single male among the progeny of an X-rayed female. All the bristles and microchaeta are considerably reduced both in length and in diameter. Since this description appears to correspond to a similar mutation described in Drosophila melanogaster by Morgan, Bridges, and Sturtevant ( 1925 ), it has been given the name provisionally.

Four sex-linked genes located in the right arm of the X chromosome were used in testing the linkage of tiny-bristle. These genes are sepia ( se, sepia eye-colour; locus, 116 ), lanceolate ( ll, lanceolate wing-shape; locus, 140 ), snapt ( sp, broken second wing-vein ; locus, 157 ), and tilt ( tt , tilted end of wing with gaps in the longitudinal veins ; locus, 182 ). The data obtained show that approximately 50% of recombination occurs between tb and all of the four genes. This indicates that the gene tb is located in the opposite arm of the X chromosome, the left arm. In order to find the exact locus of tb, further experiments are in progress. The data obtained in the first breeding tests may be considered valuable as giving more details regarding the linkage relationship of the other genes. Hence they are given in tables 5 and 6.

Table 5

Recombination data from males of the cross

$$\frac{tb}{se\ 11\ sp}\ ♀ \times \frac{+\ +\ +\ +}{}\ ♂$$ ( 12 cultures )

| Types of crossing-over | Loci of c. o. | Class | No. of ♂♂ | No. of ♀♀ |
|---|---|---|---|---|
| Non- | | se 11 sp | 134 | |
| | | tb | 177 | |
| double | tb/se, se/11 | se tb | 76 | |
| | | 11 sp | 64 | |
| single | 11/sp | se 11 | 45 | |
| | | tb sp | 55 | |
| double | tb/se, 11/sp | tb se 11 | 37 | |
| | | sp | 62 | |
| single | tb/se | tb se 11 sp | 125 | |
| | | + + + + | 228 | |
| double | se/11, 11/sp | se sp | 4 | |
| | | tb 11 | 0 | |
| single | se/11 | se | 92 | |
| | | tb 11 sp | 53 | |
| triple | tb/se, se/11, 11/sp | 11 | 6 | |
| | | se sp tb | 4 | |
| | | Total | 1162 | 1292 |

Recombination

tb/se, 51.8% se/11, 25.7%

tb/11, 51.7% 11/sp, 18.3%
( not allowing for double crossing-over )

tb/sp, 52.2%
( not allowing for double crossing-over )

Table 6

Recombination data from males of the cross

$\frac{tb}{ll\ tt}$ ♀ X $\frac{+\ +\ +}{+\ +\ +}$ ♂ ( 15 cultures )

| Types of crossing-over | Loci of c. o. | Class | No. of ♂♂ | No. of ♀♀ |
|---|---|---|---|---|
| non- | | $\frac{ll\ tt}{tb}$ | 234<br>322 | |
| single | tb/ll | $\frac{tb\ ll\ tt}{+\ +\ +}$ | 239<br>311 | |
| double | tb/ll, ll/tt | $\frac{tb\ ll}{tt}$ | 141<br>162 | |
| single | ll/tt | $\frac{ll}{tb\ tt}$ | 162<br>176 | |
| | | Total | 1746 | 1796 |

Recombination

tb/ll, 48.8%

tb/tt, 50.3% ( not allowing for double crossing-over )

ll/tt, 36.7%

## The Second Chromosome

### ( 1 ) Light ( li )

This character originated in a culture of flies carrying the sex-linked recessive vermilion ( locus, 72 ). It produces a slight dilution of the vermilion eye-colour, and hence is a modifier of vermilion. In order to test its effect in the absence of vermilion, a light-vermilion female was crossed to a wild-type male, and the $F_1$ females ( $\frac{li \quad v}{+ \quad +}$ ) were backcrossed to light vermilion males. The offspring of this backcross fall into four classes of equal numbers, namely: vermilion, wild-type, light-vermilion, and light. The eyes of flies in the last class are only slightly brighter than wild-type and therefore difficult to distinquish in the absence of vermilion.

The complete segregation of the above characters in the backcross shows that light is independent in its linkage relationship from vermilion. Tests for its linkage group were made by two crosses:

(1) Light-vermilion females to Ba or Cy ( bare body without bristles, dominant, second chromosome— locus, 40; orange-coloured eyes, recessive, third chromosome— locus, 0; curly wings, dominant, fourth chromosome — locus, 0 ) males , and backcrossing the $F_1$ males and females to light-vermilion;

(2) Light-vermilion males to Ba or Cy females and intercrossing the $F_1$ males and females. From the first cross vermilion males and females would be obtained half of which would be light-vermilion so that recombination with Ba and Cy would be easily detectable in this group if it occurred, since the classification of light is facilitated in the presence of vermilion. The purpose of the second cross ( light-vermilion♂ X Ba or Cy♀ ) was to avoid the production of vermilion females in the $F_2$ , as these would be indistinguishable from orange. Hence all females found in this $F_2$ with an eye-colour similar to light-vermilion would be known to be in reality light-orange and would indicate recombination of light with the third chromosome group( represented by orange ).

It was found in the female offspring of the second cross that orange actually gave recombination with light, which indicates that light is not on the orange chromosome. In the male backcross of the first cross, there were found quite a number of flies which were both Curly and light, but no flies which were both Bare and light; which shows that light is not on the Curly chromosome but on the Bare chromosome. In the female backcross of of the first cross, the number of crossovers ob-

tained indicated that the distance between Bare and light was considerable.

To localize more accurately the position of light, it was mated to Eyeless$_2$ ( Ey$_2$, reduced eyes; locus, 50 ) and Stubble ( Sb, short thick bristles, curved legs, cramped general appearance; locus, 82 ). Light-vermilion females were again used, and calculations were made only on the classes which included vermilion. This method has the advantage of reducing the error of observation but the disadvantage of reducing the total number of flies counted by half and so perhaps introduces an error due to smallness of numbers. The results of the two crosses are given in table 7.

Table 7

(a) Recombination data from the cross

$$\frac{Sb}{li} \Big| \frac{+}{v} ♀ \times \frac{li}{li} \Big| \frac{v}{-} ♂ \quad (\text{12 cultures})$$

| | Class | No. of ♂♂ | No. of ♀♀ | Total |
|---|---|---|---|---|
| | Sb and Sb li | 274 | 269 | 543 |
| | + + and li | 223 | 227 | 450 |
| | Total | | | 993 |
| Non-crossovers | Sb v | 222 | 226 | 448 |
| Non-crossovers | li v | 191 | 212 | 403 |
| Crossovers | Sb li v | 28 | 8 | 36 |
| Crossovers | v | 10 | 27 | 37 |
| | Total | | | 924 |

Recombination

Sb/li, 7.8%

(b) Recombination data from the cross

$$\frac{Ey_2}{li} \Big| \frac{+}{v} ♀ \times \frac{li}{li} \Big| \frac{v}{-} ♂ \quad (\text{15 cultures})$$

| | Class | ♂♂ | ♀♀ | Total |
|---|---|---|---|---|
| | $Ey_2$ and $Ey_2$ li | 280 | 280 | 560 |
| | + + and li | 289 | 287 | 576 |
| | Total | | | 1136 |
| Non-crossovers | $Ey_2$ v | 144 | 138 | 282 |
| Non-crossovers | li v | 159 | 186 | 345 |
| Crossovers | $Ey_2$ li v | 115 | 117 | 232 |
| Crossovers | v | 112 | 141 | 253 |
| | Total | | | 1112 |

Recombination

$Ey_2$/li, 43.6%

From the above data, it is seen that the recombination percentage between light and Stubble is 7.8 and that between $Eyeless_2$ and light is 43.6. The position of Stubble has been placed at 82 by Donald ( 1936 ). The recombination data indicate that the locus of light is right to that of Stubble and its position is 89 units down the chromosome map. The recombination data between $Eyeless_2$ and light strongly suggest that the map distance between $Eyeless_2$ and Stubble is greater than was calculated by Donald. A more exact analysis regarding the relative positions of $Eyeless_2$ and Stubble will be necessary to determine their relationship.

( 2 ) Balancers down ( bd )

This mutation was found among the $F_2$ offspring of an X-rayed female. It shows itself as a downward bending of the balancers which in extreme cases are turned right toward the abdomen, with the knobs close to the sides of the body. Both balancers are usually affected, but sometimes one balancer is turned right down while the other is held at an angle. The character is probably very variable, and there is some overlapping with wild-type. In working with this character, it is essential that the flies be not over-etherized, for then the wings become erected and lie toward the back, which causes a bending of the balancers , making them project at a considerable angle toward the abdomen and thus rendering the wild-type indistinguishable from the mutant.

A preliminary test was made with Ba or Cy ( bare body without bristles, dominant, second chromosome — locus, 40; orange-coloured eyes, third chromosome— locus, 0, recessive; curly wings, dominant, fourth chromosome— locus, 0 ). A Ba or Cy female was mated to a bd male and the $F_1$ males and females were backcrossed to balancers down. Also, crosses were made between the $F_1$ males and females. It was found in the cross $F_1♀ \times F_1♂$ that balancers

down gave recombination with orange, which shows that bd is not on the orange chromosome. In the male backcross, it was found that bd gave no recombination with Ba but gave recombination with Curly, which shows that bd is on the Ba chromosome. In the female backcross, the following recombination data were obtained, Curly being disregarded ( table 8 ):

Table 8

Recombination data from the cross

$\frac{\text{Ba or Cy}}{\text{bd}}$ ♀ X $\frac{\text{bd}}{\text{bd}}$ ♂ ( 6 pair matings )

| | Class | No. of ♂♂ | No. of ♀♀ | Total |
|---|---|---|---|---|
| Non-crossovers | Ba | 202 | 229 | 431 |
| | bd | 205 | 176 | 381 |
| Crossovers | Ba bd | 44 | 36 | 80 |
| | + + | 59 | 69 | 128 |
| | Total | | | 1020 |

Recombination

Ba/bd, 20.3%

Table 9

Recombination data from the cross

$\frac{Ba}{Ey_2}$ bd♀ X $\frac{bd}{bd}$♂ ( 24 pair matings ) .

| Types of crossing-over | Loci of c. o. | Class | No. of ♂♂ | No. of ♀♀ | Total |
|---|---|---|---|---|---|
| non- | | $\frac{Ey_2}{}$ | 954 | 1063 | 2017 |
| | | $\frac{Ba\ bd}{}$ | 1039 | 1092 | 2131 |
| single | $Ey_2$/bd | $\frac{Ba}{}$ | 49 | 101 | 150 |
| | | $\frac{Ey_2}{}$ | 68 | 95 | 163 |
| single | Ba/$Ey_2$ | $\frac{Ba\ Ey_2}{}$ | 98 | 102 | 200 |
| | | $\frac{bd}{}$ | 124 | 116 | 240 |
| double | Ba/$Ey_2$, $Ey_2$/bd | $\frac{Ba\ Ey_2\ bd}{}$ | 13 | 8 | 21 |
| | | $\frac{+\ +\ +}{}$ | 5 | 15 | 20 |
| | | Total | | | 4942 |

Recombination

Ba/$Ey_2$, 9.7%

$Ey_2$/bd, 7.1%

Ba/bd, 16.8% ( allowing for double crossing-over )

This gave the distance of balancers down as 20.3 units from Bare. A three-point experiment was next made in order to determine the position of bd with respect to $Ey_2$ which has been located at 10 units from Ba ( Auerbach, unpublished ). Females of the constitution $\frac{Ba \quad bd}{Ey_2}$ were mated to bd males. The results are given in table 9.

From these data, it is seen that bd is 17 units from Ba and 7 units from $Ey_2$ . Since $Ey_2$ is 10 units from Ba, the sequence of the genes must be Ba $Ey_2$ bd. Ba has been placed at 41.5 and $Ey_2$ at 51.9 ( Auerbach, unpublished ), therefore bd must be placed at 59.

The discrepancy between the distances of bd from Ba ( 20.3 and 17.0 ) as found in the two experiments is presumably due to the fact that in the first cross the flies were sometimes over-etherized and classification was in consequence probably less accurate than in the second cross when special care was taken to avoid this danger.

## The Fourth Chromosome

### ( 1 ) White tip ( wt )

Flies with this character first had their origin from X-rayed cultures but were later also observed in non-treated cultures. It may therefore be a spontaneous gene mutation. Its effect is to whiten all the bristles for about half or two thirds of the length from the tip. The mutant can best be separated from wild-type by turning the animal on its side with its back toward the light , and observing the bristles on the scutellum. Ordinary wild-type flies will show bristles which are quite black, but the bristles of the mutant will present a glistening transparent appearance . The identification , however, has to be made when the fly is still quite young, for otherwise the white tip becomes easily broken off and the separation from wild-type becomes difficult.

A cross to Ba or Cy ( bare body without bristles, dominant, second chromosome —locus, 40 ; orange-coloured eyes, recessive, third chromosome— locus, 0 ; curly wings, dominant, fourth chromosome — locus, 0 ) with white tip as the mother gave an $F_1$ none of the sons or daughters of which had white tip, showing that the character is an autosomal recessive. In $F_2$ white tip was found

to show recombination with orange, while in the male backcross it showed recombination with Bare but not with Curly. White tip was thus concluded to be on the Curly chromosome. In the female backcross, a large amount of crossing-over with Curly was observed, which indicates that white tip is at a considerable distance from the locus of Curly. In order to find the more exact position of white tip, four linkage experiments were made.

(1) White tip with blunt tangled white tip( bl , blunt scutellum, locus— 16 units from tangled; tg, fused second and third longitudinal veins at their distal ends, locus— 0 ),

(2) White tip with tangled white tip,

(3) White tip with tangled short$_4$ white tip ( $s_4$, shortening of the fourth and fifth longitudinal veins with elimination of the posterior crossvein ,locus— 57 ),

(4) White tip with short$_4$.

Blunt, tangled, and short$_4$ are the known genes on the Curly chromosome. Jaunty ( j, slight upturning of the tip of the wing, locus—44 ) is another gene on the Curly chromosome ( Crew and Lamy,1935 ) but a trial with jaunty failed owing to the fact that the two genes could not be combined , which suggests that they may lie close together. The

Table 10

Recombination data from the cross

$\frac{bl\ tg}{wt}$ ♀ X $\frac{bl\ tg\ wt}{bl\ tg\ wt}$ ♂ ( 14 pair matings )

| Types of crossing-over | Locus of c. o. | Class | No. of ♂♂ | No. of ♀♀ | Total |
|---|---|---|---|---|---|
| non- | | bl tg | 154 | 188 | 342 |
| | | wt | 183 | 147 | 330 |
| single | tg/wt | bl tg wt | 161 | 168 | 339 |
| | | + + + | 169 | 182 | 351 |
| single | bl/tg | bl wt | 36 | 24 | 60 |
| | | tg | 65 | 96 | 161 |
| double | bl/tg, tg/wt | bl | 32 | 29 | 61 |
| | | tg wt | 48 | 69 | 117 |
| | | Total | | | 1761 |

Recombination

bl/tg, 22.6%

tg/wt, 49.2%

bl/wt, 51.7% ( not allowing for double crossing-over )

Table 11

Recombination data from the cross $\frac{tg}{wt}$ ♀ X $\frac{tg\ wt}{tg\ wt}$ ♂

( 25 pair matings )

| | Class | No. of ♂♂ | No. of ♀♀ | Total |
|---|---|---|---|---|
| Non-crossovers | tg | 457 | 465 | 922 |
| | wt | 477 | 490 | 967 |
| Crossovers | tg wt | 446 | 459 | 905 |
| | +++ | 512 | 491 | 1003 |
| | Total | | | 3797 |

Recombination,

tg/wt, 50.6%

Table 12

Recombination data from the cross

$$\frac{tg \quad s_4}{wt} \text{ ♀ } \times \frac{tg\ wt\ s_4}{tg\ wt\ s4} \text{ ♂ } ( 18 \text{ cultures } )$$

| Types of crossing-over | Loci of c. o. | Class | No. of ♂♂ | No. of ♀♀ | Total |
|---|---|---|---|---|---|
| non- | | tg s4 | 189 | 273 | 462 |
| | | wt | 306 | 304 | 610 |
| double | tg/wt, wt/$s_4$ | tg wt $s_4$ | 15 | 37 | 52 |
| | | + + + | 53 | 53 | 106 |
| single | tg/wt | tg wt | 270 | 287 | 557 |
| | | $s_4$ | 274 | 324 | 598 |
| single | wt/$s_4$ | tg | 32 | 37 | 69 |
| | | $s_4$ wt | 33 | 36 | 69 |
| | | Total | | | 2523 |

Recombination

tg/wt, 52.3%

wt/$s_4$, 11.4%

tg/$s_4$, 51.2% ( not allowing for double crossing-over )

Table 13

Recombination data from the cross $\frac{wt}{s_4}$ ♀ X $\frac{wt\ s_4}{wt\ s_4}$ ♂

( 14 pair matings )

| | Class | No. of ♂♂ | No. of ♀♀ | Total |
|---|---|---|---|---|
| Non-crossovers | $\frac{s4}{}$ | 380 | 440 | 820 |
| | $\frac{wt}{}$ | 403 | 404 | 807 |
| Crossovers | $\frac{wt\ s4}{}$ | 45 | 52 | 97 |
| | $\frac{+\ +}{}$ | 69 | 61 | 130 |
| | Total | | | 1854 |

Recombination

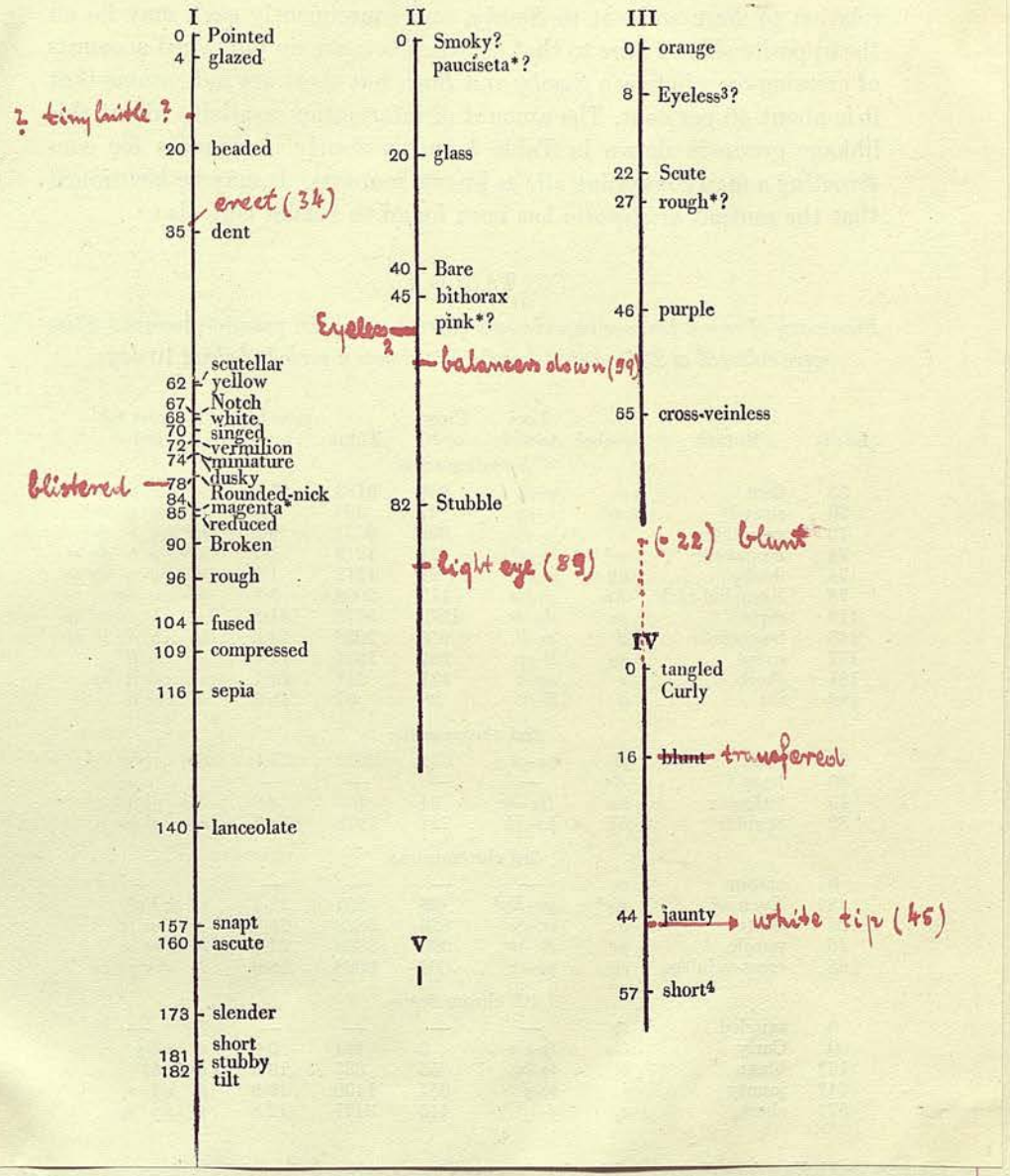
$wt/s_4$, 12.2%

results for the four crosses made are given in tables 10, 11, 12, 13.

The data indicate that the sequence of the genes is probably: $\underline{s_4}$, wt, tg, bl. Taking $\underline{s_4}$ as zero ( Tan, 1936 ), wt must be at twelve. This conclusion is supported by our failure to obtain jaunty white tip flies, since jaunty according to Tan is at 17, which would be no more than five units from white tip. And if the position of jaunty as given by Donald ( 1936 ) is more correct( at 13 ), the two genes must be even closer together.

Since white tip gives approximately 50% recombination both with tangled and with blunt, and blunt gives 22% recombination with tangled, the only indication that tangled is between blunt and white tip and not on the distal side of blunt, is the fact that the classes bl and tg wt in cross 1 ( table 10 ) are represented by significantly fewer flies than the other crossover classes, and hence may be presumed to be double crossovers. Also, to fit in with the assumption made above regarding the relative positions of jaunty and white tip , the classes tg wt $\underline{s_4}$ and + + + have been considered as double crossover classes in cross 3 ( table 12 ) in spite of the fact that the number of flies in these classes is slightly greater than in the

classes tg and $s_4$ wt. However, the distances are too great for this interpretation to be more than tentative.




Corrected chromosome maps of Drosophila pseudo-obscura according to the data given in the present paper.

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Summary

1. The following six mutations, bli, er, tb, li, bd, and wt , are described. Bli and er affect the wings, tb and wt affect the bristles, li affects the eyes, and bd affects the balancers.
2. Bli, er, and tb belong to the first linkage group. Their loci are as follows: bli, 78; er, 34 ; tb is in the left arm.
3. Li, and bd belong to the second linkage group. Their loci are : li, 89; bd, 59.
4. Wt belongs to the fourth linkage group. Its locus is 12.‡

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‡ Chromosomes I, II, III are based on Donald's data 1936 ) while chromosome IV is based on Tan's(1936 ), $s_4$ being taken as 0 instead of Curly as in Donald's map.

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